Human tendon adaptation in response to mechanical loading

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Zusammenfassung

Sehnen übertragen die vom Muskel erzeugten Kräfte auf das Skelett. Die Eigenschaften der Sehne beeinflussen die Leistungen des Muskels und damit unsere alltäglichen Bewegungen sowie athletische Leistungsfähigkeiten. Weiterhin reagieren Sehnen auf mechanische Belastungen durch eine Adaptation ihrer mechanischen, morphologischen und Materialeigenschaften. Allerdings sind die Effekte spezifischer Parameter des mechanischen Dehnungsstimulus sowie allgemeiner Belastungsbedingungen auf die Sehnenanpassung nicht vollständig geklärt. Die vorliegende Arbeit vertieft den derzeitigen Kenntnisstand über die Auswirkungen mechanischer Belastungen auf die Anpassung der menschlichen Sehne.

Das adaptive Potential der Sehne wurde durch einen Vergleich der Sehneneigenschaften zwischen dem dominanten und nicht dominanten Bein, in Bezug auf seitenabhängige Belastungen, experimentell untersucht. Um den Effekt verschiedener interventionsinduzierter Belastungen auf das Ausmaß der Sehnenanpassung zu bestimmen, wurde zudem ein systematischer Literaturreview nebst Metaanalyse durchgeführt. Der Einfluss spezifischer Parameter des mechanischen Dehnungsstimulus (Rate und Dauer) auf die Sehnenanpassung wurde mittels zweier Trainingsinterventionen untersucht. Magnetresonanztomographie, Ultrasonographie und Dynamometrie dienten der Quantifizierung der Sehneneigenschaften.

Der Vergleich zwischen den Achillessehneneigenschaften des dominanten und nicht dominanten Beins zeigte einen signifikanten Unterschied des Young's Modulus (Materialeigenschaften), mutmaßlich eine Konsequenz seitenabhängiger Alltagsbelastungen beider Beine (Fußdominanz). Die Metaanalyse ermittelte hohe Effektgrößen der inkludierten Interventionen auf die mechanischen und Materialeigenschaften sowie eine niedrige Effektgröße auf die morphologische Sehnencharakteristik. Die Unterschiede in den Belastungen der einzelnen Studien hatten einen Einfluss auf die adaptiven Reaktionen, wobei hohe Intensitäten einen gesteigerten Effekt zeigten. Die beiden Interventionen belegten, dass eine hohe Dehnungsrate und eine lange Dauer keinen übergeordneten Stimulus zur Sehnenanpassung im Vergleich zu einer hohen Dehnungsmagnitude und niedrigen Dehnungsfrequenz darstellen.

Die Experimentalstudien sowie die Literaturanalyse bestätigen, dass Sehnen auf ihre mechanischen Konstellationen durch eine Adaptation ihrer Eigenschaften reagieren. Weiterhin zeigen die Ergebnisse der Querschnittstudie und Metaanalyse, dass Materialeigenschaften sensitiver gegenüber Belastungsänderungen sind und im Vergleich zur Morphologie zeitiger adaptieren. Unterschiede in den Belastungen beeinflussen dabei signifikant die Magnitude der Adaptation. Die Ergebnisse der Interventionen in Kombination mit früheren Studien lassen den Schluss zu, dass eine hohe Dehnungsmagnitude, eine adäquate Dauer und repetitive Belastung essentiell für einen effektiven Stimulus sind. Die Ergebnisse liefern wichtige Erkenntnisse bezüglich einer Verbesserung von Sehneneigenschaften im Kontext der athletischen Leistung sowie Verletzungsprävention und -rehabilitation.

Abstract

Tendons are connective tissue and transmit the force exerted by a muscle to the skeleton. The properties of tendons influence the muscle output and, therefore, affect human daily locomotion as well as athletic performances. Furthermore, tendons respond to mechanical loading by changing their mechanical, material and morphological properties. However, the effect of specific parameters of the mechanical strain stimulus as well as general loading conditions on tendon adaptation is not completely understood. The present thesis aims to deepen the current knowledge about the mechanical conditions that may affect the human tendon adaptive responses in vivo.

Tendon adaptive responses were experimentally investigated by means of a comparison of tendon properties between the non-dominant and dominant leg of normally active individuals to assess the potential effect of side-dependent loading pattern. Furthermore, a systematic literature review and meta-analysis was applied to examine the effect of various intervention-induced mechanical loading conditions on the magnitude of tendon adaptive responses. To investigate the effect of specific parameters of the mechanical strain stimulus (rate and duration) on tendon adaptation, two controlled exercise interventions (14 weeks/4 times a week) were conducted. A combination of magnetic resonance imaging, dynamometry and ultrasonography was used to assess the tendon properties in the experimental studies.

The comparison of the Achilles tendon properties between the non-dominant and dominant legs revealed a significant difference of the Young's modulus (i.e. material properties), likely a result of side-dependent daily loading pattern of both legs (i.e. foot/leg dominance). Furthermore, the meta-analysis revealed high intervention effect sizes on the tendon mechanical and material properties and a low effect size on the morphological property. Differences in the various loading conditions between studies notably affected the tendon adaptive responses, indicating e.g. a key role of high loading intensities. The two exercise interventions showed that a higher strain rate and longer strain duration did not provide a superimposed effect for tendon adaptation compared to high strain magnitude and low strain frequency.

In conclusion, the experimental and comprehensive literature analysis approach revealed that tendons respond to their mechanical environment and adapt through alterations of their properties. As indicated by the findings of the cross-sectional study and meta-analysis, the material properties seem to be more sensitive and quicker in response to changes in the mechanical loading conditions compared to the morphological properties and that variations in the loading conditions significantly affect the magnitude of the adaptation. The results of the two interventions, in combination with earlier studies, suggest that a high strain magnitude, an appropriate strain duration and repetitive loading are essential for an effective stimulus for tendons. These findings provide valuable information with regard to the improvement of tendon properties in the context of athletic performance as well as injury prevention and rehabilitation.

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1. Introduction and literature review

The following introduction summarises fundamental aspects of recent tendon research. At first, the tendon structure as a multi-unit hierarchical organization and the cellular and molecular composition of tendon tissue are presented. Thereafter, the functional interaction between tendon and attaching muscle is discussed, considering the effect of the tendon mechanical properties on the muscle output. Particular attention is given to the plasticity of tendons to chronic mechanical loading in a subsequent chapter. In the final section, methodological considerations for the measurement and analysis of tendon's mechanical, material and morphological properties in vivo will be reviewed. Based on this information the purpose of the present thesis is formulated.

1.1 Structure and composition of tendons

1.1.1 Tendon structure

Tendons are interposed between muscles and bones. They connect the muscle to the bone, most often directly distal to the joint on which the respective muscle principally acts and, thus, allow for joint movements (Benjamin and Ralphs, 1997). Tendons feature a wide range of shapes and sizes, depending on the characteristics of the respective muscle and bone (Józsa and Kannus, 1997). The part of the tendon, which links the muscle to the bone refers to the free or external tendon whereas the aponeurosis or internal tendon provides the attachment area for the muscle fibres (Magnusson et al., 2008; Nigg and Herzog, 1999).
Regarding the histology, tendons are characterized by a multi-unit hierarchical structure (fig. 1.1) (Benjamin and Ralphs, 1997; Elliott, 1965; Kastelic et al., 1978). The smallest structural unit is the fibril, consisting of a quarter-staggered arrangement of aligned collagen molecules (i.e. each molecule overlaps its neighbour by a quarter of its length). Collagen molecules are long and the stiff rods, containing three helical polypeptide alpha-chains that wind around each other to form a triple helix, extend throughout the length of the molecule (Józsa and Kannus, 1997). Collagen fibres that are composed of collagen fibrils form the next level of tendon structure (fig. 1.1). The

endotenon, a thin layer of connective tissue that includes blood vessels, lymphatics and nerves, binds several collagen fibres to a primary fibre bundle (sub fascicle) (fig. 1.1). Primary fibre bundles form to secondary fibre bundles (fascicles) and then to tertiary fibre bundles. Each fascicle level is again enclosed by endotenon tissue. The final structural level is the tendon unit, as the conjunction of the tertiary fibre bundles (fig. 1.1). The tendon unit is surrounded by the epitenon, a connective tissue that also provides vascular, lymphatic and nerve supply to the tendon. A third surrounding connective tissue layer, the paratenon, is connected with the epitenton to the so-called peritenon (fig. 1.1). It has been suggested that this layer reduces the friction with adjacent tissues (Benjamin and Ralphs, 1997; Kastelic et al., 1978; Wang, 2006). All levels in this hierarchical structure are aligned parallel to the axis of the tendon (Elliott, 1965), allowing for an optimal mechanical load transmission (Wang et al., 2012). In an unloaded condition, the collagen fibres are characterized by a crimping or wavy formation (crimp pattern), most likely due to proteoglycan fibre cross-linking (see next chapter). However, when the tendon is stretched the wavy configuration disappears in correspondence to the straightening of the collagen fibres (Elliott, 1965; Józsa and Kannus, 1997).

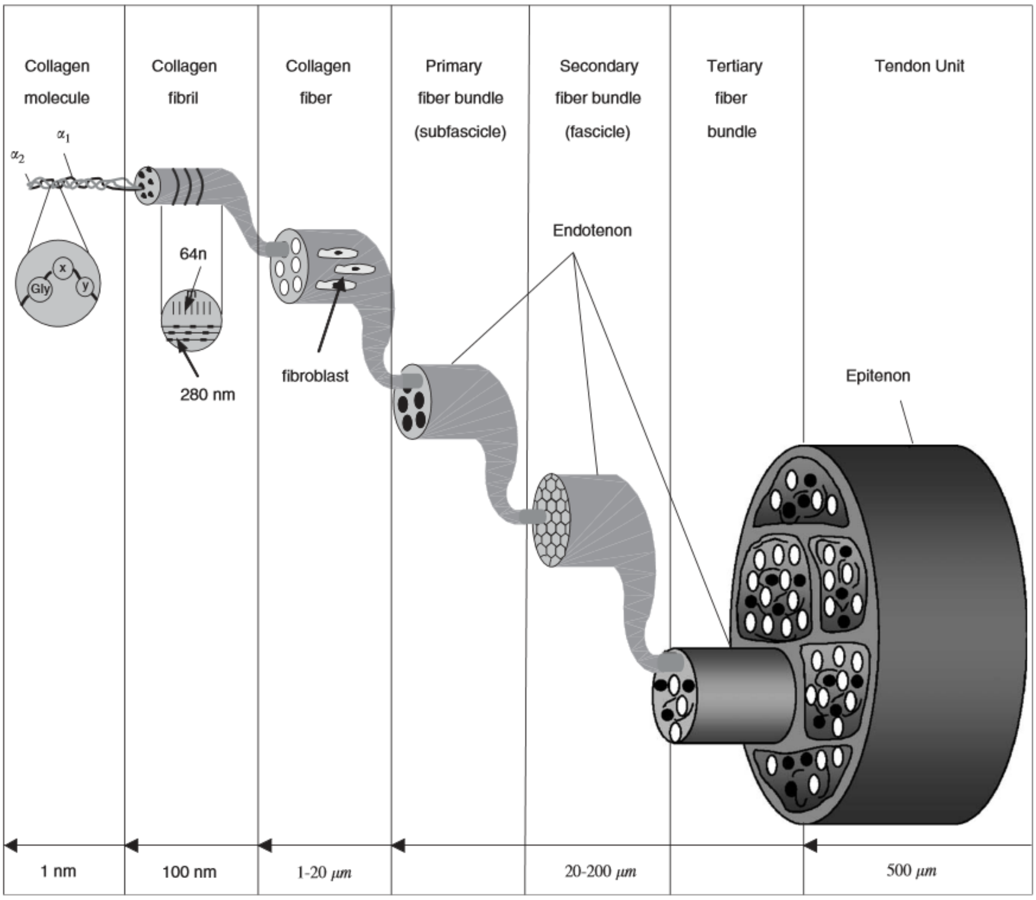

Fig. 1.1 Schematic illustration of the multi-unit hierarchical structure of the tendon. (Wang 2006, J. Biomech., 39:1563-1582, p.1565; with permission by Elsevier)

At their ends, tendons connect to bone and muscle. The enthesis, as the tendon-bone junction, consist of either a fibrous or a fibro-cartilaginous connection type. Whereas at the fibrous enthesis the tendon directly attaches to the bone, the fibro-cartilaginous enthesis features a transitional zone of hyaline fibrocartilage to distribute mechanical loads (Benjamin et al., 2002). The myotendinous junction connects the muscles to the tendon by a special arrangement of the terminal myofibrils (Benjamin and Ralphs, 1997). For example, membrane folding of the terminal muscle cells increases the junction surface area, myofilament bundling at the terminal processes of muscle cells and also out-branches of smaller myofibrils allow for the insertion of the tendon collagen fibrils on the muscle and, thus, for the transmission of the muscular forces (Michna, 1983; Tidball, 1991; Wang, 2006).

1.1.2 Tendon composition

Tendons primarily consist of cells, collagens, proteoglycans, glycoproteins and water (Silver et al., 2003). Collagen is the most abundant protein in the tendon extracellular matrix with type I collagen being the major component. Type I collagen constitutes about 60% of the tendon dry mass and about 95% of the total collagen content in tendons. Collagen type III and V make up the remaining 5%, where type III is mainly located in the endotenon and epitenon and type V in the core of type I collagen fibrils (Wang, 2006). Collagen type II, VI, IX, X and XI quantities are minor and primarily located in the enthesis (Fukuta et al., 1998). Type I collagen mainly forms parallel fibres (longitudinal) but is also organized horizontally and transversely. Furthermore, collagen fibres crossing each other form spirals and plaits along their course (Józsa and Kannus, 1997). It has been suggested that the vast majority in content, the organization and characteristic of collagen type I play a key role for the tensile strength of tendons (Wang et al., 2012). The collagen in the extracellular matrix is intra- and intermolecular cross-linked via enzymes (e.g. lysyl oxidase) and non enzymic glycation (Avery and Bailey, 2005; Bailey et al., 1998; Eyre et al., 1984; Reiser et al., 1992), which influences the tendon mechanical properties (i.e. Young’s modulus, Thompson and Czernuszka, 1995). Beside collagen, proteoglycans (e.g. aggrecan and decorin) and glycoproteins (e.g. tenascin-C, fibronectinand elastin) account for important functions in the tendinous tissue (Halper and Kjaer, 2014; Silver et al., 2003; Wang et al., 2012). While aggrecan binds water (50 times their weight) and therewith resists compression and shear, decorin allows for collagen fibrillar slippage during mechanical deformation. Glycoproteins not only contribute to the mechanical stability of tendinous tissue through its interaction with collagen fibrils (tenascin-C) but also facilitate wound healing (fibronectin). Elastin in particular accounts for the length recovery following mechanical stretching (i.e. elastic tendon properties) (Halper and Kjaer, 2014; Józsa and Kannus, 1997; Pins et al., 1997; Wang et al., 2012). Tendons further contain different types of cells, i.e. tendon cells (tenocytes and tenoblasts), chondrocytes, synovial cells and vascular cells. The tenocytes and tenoblasts are the majority of type of cells in tendons and are located between collagen fibre bundles, aligned to the axis of the tendon. Tendon cells interact with the extracellular matrix via cell-matrix coupling and their main function is to synthesize extracellular matrix components (i.e. collagen, fibronectin and proteoglycans) in order to maintain tendon homeostasis. Collagen is synthesized in the tendon cells as so-called tropocollagen and then secreted into the matrix as procollagen (Kjaer et al., 2009; Wang et al., 2012; Wang and Thampatty, 2006). More recently, tendon stem cells were identified as a new cell type in human tendons. Stem cells possess clonogenicity, multipotency and self-renewal properties and, therefore, may be essential for tendon maintenance and repair (Bi et al., 2007; Yin et al., 2010).

1.2 Tendon mechanics

The present chapter summarises the main functions of tendons as well as their mechanical properties, and how these affect the interaction of muscle and tendon with regard to human locomotor performance.

1.2.1 Tendon function

The main function of tendons is to transmit the force exerted by the attached muscle to the bone and, therefore, allow for joint and limb movements (Józsa and Kannus, 1997; Magnusson et al., 2003). Tendons possess great tensile strength due to the molecular and submolecular structure of collagen, the main structural component of tendons (see also previous chapter). This strength enables tendons to transmit the muscle forces with minimal energy exchange and deformation (Józsa and Kannus, 1997; Nigg and Herzog, 1999). On the other hand, tendons are flexible due to their elastic fibre content and, therefore, rest length is recovered following loading-induced deformation (Elliott, 1965; Józsa and Kannus, 1997). It is this spring-like characteristic that allows for a dynamic mechanical interaction between the muscle and tendon (Magnusson et al., 2003). These important functions will be discussed in the next chapter in more detail. Furthermore, the flexibility of the tendon at their insertion and origin, enables an alignment of the tendon to the different directions of the acting muscles forces (e.g. at different joint angles) (O'Brien, 1992).

Several secondary functions of tendons have been mentioned in the literature, e.g. elimination of the need for unnecessary length of the muscle between origin and insertion, bending at joints, absorption of sudden shocks and the sensation of proprioceptive stimuli via mechanoreceptors (e.g. golgi tendon organs, Ruffini corpuscles, Vater-Pacini corpuscles and free nerve endings) (Józsa and Kannus, 1997; Magnusson et al., 2003).

1.2.2 Mechanical properties of tendons

The mechanical properties of tendons can be assessed by means of loading tests. In vitro, single tendinous fibres are constantly elongated and the corresponding tensile force is recorded (Butler et al., 1978). The most common procedure for human in vivo measurements includes a maximum voluntary muscle contraction on a dynamometer and the corresponding tendon elongation is visualized using ultrasonography (more detailed in chapter 1.4) (Fukashiro et al., 1995; Kubo et al., 1999). The loading test paradigm allows to determine the relationship of tendon force to tendon elongation (fig. 1.2) and therewith the tendon mechanical properties. The tendon structure and composition described in the previous chapter directly affects the force-elongation relationship (Silver et al., 2003). At rest, a crimp pattern of the collagen fibres and fascicles can be observed, which disappears following the application of tensile forces in correspondence to the straightening of the fibres (Elliott, 1965; Hess et al., 1989; Józsa and Kannus, 1997). Since the initial forces are accompanied by a pronounced tendon elongation, the primary part of the force-elongation relationship is concave-shaped and was termed toe region (fig. 1.2) (Butler et al., 1978; Elliott, 1965). Increasing the tendon force further, the elongation shows a relatively linear response (fig. 1.2) (Butler et al., 1978; Elliott, 1965). The slope of this linear region of the force-elongation relationship was defined as tendon stiffness (Butler et al., 1978; Heinemeier and Kjaer, 2011). Up to the end of this region the tendon elongation is fully recovered to rest length when the load is removed (i.e. reversible strain) (Józsa and Kannus, 1997). At the terminal part of the linear region, micro failure of single collagen fibres may occur, indicating the so-called yield region as the third part of the force-elongation relationship. Relatively low force increments are now accompanied by massive elongation (Nigg and Herzog, 1999). Consequently, additional fibres fail and fibre cross-links are detached until macroscopic failure takes place and the load-supporting ability of the tendon is lost (fig. 1.2) (Butler et al., 1978; O'Brien, 1992).

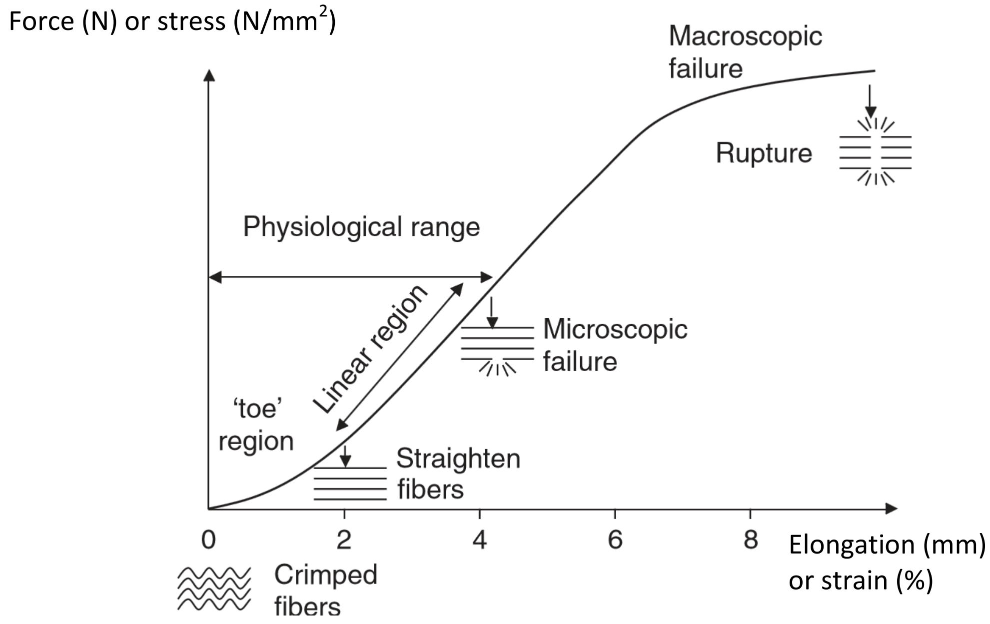

Fig. 1.2 Schematic stress-strain and force-elongation relationship. (Modified from Wang 2006, J. Biomech., 39:1563-1582, p.1567; with permission by Elsevier)

The tendon force-tendon elongation relationship is directly affected by the morphological tendon properties, i.e. cross-sectional area and rest length (Butler et al., 1978). Accordingly, a thicker and/or shorter tendon accounts for a steeper slope of the force-elongation relationship, indicating less tendon elongation at a given tendon force and, thus, a higher stiffness of the tendon (Butler et al., 1978; Thompson and Czernuszka, 1995). To account for the effect of cross-sectional area and length, the tendon force can be normalized to the tendon cross-sectional area (i.e. tendon stress) and the tendon elongation to the tendon rest length (i.e. tendon strain) (Butler et al., 1978; Heinemeier and Kjaer, 2011). The resulting tendon stress-tendon strain relationship is quite similar in shape to the force-elongation curve, but independent of the individual tendon morphology (fig. 1.2) (Butler et al., 1978). Therefore, the stress-strain relationship displays the actual material characteristics of the tendon. The linear slope of the stress-strain relationship is referred to as Young's modulus (or elastic modulus) and is a common parameter to describe the material properties of a tendon (Arampatzis et al., 2009; Butler et al., 1978; Heinemeier and Kjaer, 2011). Accordingly, a high Young's modulus indicates a relatively stiff tendon tissue (Heinemeier and Kjaer, 2011). For the stress to strain relationship the toe-region lies typically below 3% and the linear region extends to about 4-5% of tendon strain (Nigg and Herzog, 1999; Wang, 2006). Macroscopic tendon failure was reported at strain-levels of 8-10% as investigated by in vitro tests (O'Brien, 1992; Wang, 2006). However, tests on whole tendons also indicated that higher levels of strain might be tolerable (Józsa and Kannus, 1997), most likely due to the three-dimensional organization of collagen fibre bundles throughout the tendon (Butler et al., 1978). Whereas the ultimate strain (i.e. strain at tendon failure) is more or less constant (Abrahams, 1967; LaCroix et al., 2013; Loitz et al., 1989; Nakagawa et al., 1996), the ultimate stress (i.e. stress to tendon failure) is dependent on the material properties (Józsa and Kannus, 1997; Nigg and Herzog, 1999; Thompson and Czernuszka, 1995).

Furthermore, due to the content of collagen, elastin, water and the interactions between collagenous and non-collagenous proteins (e.g. proteoglycans), tendons feature viscous and elastic properties (Wang, 2006). The elastic component allows for a recovery of the rest length following loading-induced elongation and is a time-independent phenomenon, whereas the viscous component is responsible if recovery was not complete and, by contrast, strongly depends on time (Józsa and Kannus, 1997). Viscoelasticity accounts for several specific characteristics of tendons, like force-relaxation, creep and hysteresis (Butler et al., 1978; Józsa and Kannus, 1997; Nigg and Herzog, 1999). Force-relaxation indicates that the load, which is required to maintain a certain strain-level decreases over time. Creep, on the other hand, means that tendon length increases over time during a constant load application (Butler et al., 1978; Józsa and Kannus, 1997). Viscosity is also responsible for the sensitivity of tendons to different strain rates. During lower strain rates the deformation of the tendon is higher. Thus, the tendon absorbs more strain energy but is less effective in transferring loads. With higher rates, the tendon elongates less (higher stiffness) and the load transfer becomes more efficient (Józsa and Kannus, 1997; McNeill Alexander, 2002; Noyes et al., 1974; Wren et al., 2001). During a loading cycle, characterized by a stretch and recoil of the tendon, the resulting force-elongation curve forms a loop, indicating that a proportion of strain energy expended during elongation is not completely recovered when the load is removed (Butler et al., 1978; Nigg and Herzog, 1999). This phenomenon is called hysteresis and the area between the two curves refers to the energy that is dissipated (e.g. as heat). However, the loss of the exerted energy was reported to be low (i.e. 6-11%), indicating that most energy is recovered when the applied force is removed (Bennett et al., 1986; Ker, 1981). Several in vivo measurements on human tendons reported higher hysteresis values but these discrepancies might be explained by the challenging and different methodological approaches used for tendon elongation measurement in vivo (Finni et al., 2013; Lichtwark et al., 2013). Hysteresis, force-relaxation and creep are particular examples for the viscous component of tendon properties (Finni et al., 2013; Nigg and Herzog, 1999).

1.2.3 Functional interaction of muscle and tendon

Tendon and muscle work as a unit within the musculoskeletal system, since the force generated by the muscle is transferred to the bone by the tendon. The non-rigidity of tendons considerably affects the performance capability of the corresponding muscle due to the force-length and force-velocity-relationships of the muscle fibres, as well as by the storage and return of elastic strain energy during locomotion (Ettema et al., 1990; Fukunaga et al., 2002; Hof et al., 1983; McNeill Alexander, 2002).

First, the non-rigidity of tendons influences the actual working length of the muscle fibres. Fukunga et al. (2002) reviewed several studies on different types of locomotion, providing evidence that during walking (Fukunaga et al., 2001; Hof et al., 1983), ankle bending (Kubo et al., 2000a; Sakuma et al., 2011), jumping (Ishikawa et al., 2005) and running (Lichtwark et al., 2007), tendon compliance may be responsible for a right shift of the actual working length of the sarcomers to their optimum length (i.e. plateau of the force-length relationship) (fig. 1.3). More precisely, during the first phase of the muscle contraction, the connected tendon is stretched due to its compliance. This induces the right shift of sarcomere working length on the ascending limb of the force-length curve, allowing the muscle fibres to contract nearly constantly around the plateau region (i.e. optimum length; fig. 1.3). Thus, the force potential of the muscle fibres is enhanced due to the sarcomer force-length relationship (Fukunaga et al., 2002; Hof et al., 1983).

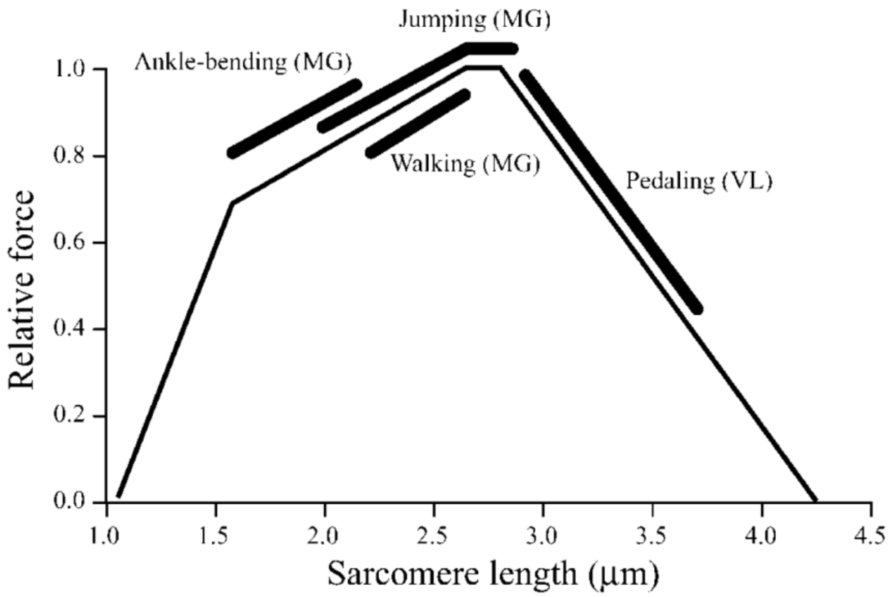

Fig. 1.3 Force-length relation of a sarcomere and sarcomere lengths during ankle bending, jumping, walking and pedaling, indicating that the tendon compliance allows the muscle fibres to contract around the plateau region of the sarcomer force-length curve (except pedaling) and, thus, enhances the force potential.(Fukunaga et al. 2002, Exerc. Sport Sci. Rev., 30:106-110, p.110; with permission by Wolters Kluwer Health)

Furthermore, during locomotion (e.g. running) the muscle-tendon unit is lengthened during load bearing (after a small shortening during initial touch down) and shortened during propulsion, while the compliant tendon takes over a part of the whole muscle-tendon length change (i.e. stretch and recoil) (Lichtwark et al., 2007; Roberts et al., 1997). Consequently, the amplitude and velocity of the muscle fibre shortening in the concentric phase is reduced and the muscle fibre contracts at a relatively lower shortening velocity compared to the velocity of the whole muscle-tendon unit. This decrease in shortening velocity is advantageous for the force potential of the muscle fibres due to the force-velocity relationship (Hill, 1938). For example, Lichtwark et al. (2007) reported that during the stance phase the gastrocnemius medialis muscle fascicles act almost isometrically in walking and with a low shortening velocity during running, which was always accompanied by an increase in the strain of the series elastic elements (Achilles tendon and aponeurosis) (Lichtwark et al., 2007). Likewise, during drop-jumps the Achilles tendon-aponeurosis compliance enabled the gastrocnemius medialis muscle to work in an isometric-concentric mode despite muscle-tendon unit lengthening, facilitating the muscle force potential (Ishikawa et al., 2005).

Furthermore, during locomotion mechanical energy can be stored briefly as elastic strain energy in the tendon and then returned during its elastic recoil. The regained energy may contribute to the propulsion phase of the movement (Alexander, 1991; Hof et al., 1983; McNeill Alexander, 2002). However, although some of the work performed during locomotion can be provided passively through the elastic strain energy storage, active muscles must provide the necessary force to support the body (Roberts et al., 1997). As described in the previous section, the tendon compliance allows the muscle to work almost isometrically, thus, producing a higher force without mechanical work being performed. In contrast, during a shortening contraction, mechanical work rate (power) increases, but the force that can be produced decreases notably (Alexander, 2000) due to the force-velocity relationship (Hill, 1938). Therefore, Roberts et al. (1997) suggested that the metabolic cost of force production during locomotion is minimized by operating the muscle fibres at slow velocities or even isometrically, while the stretch and recoil of the tendon provide the work. Due to the higher force potential during isometric conditions, less muscle fibres need to be activated compared to the shortening condition, which notably benefits locomotion economy (Alexander, 2000; Bobbert, 2001; Fletcher et al., 2013). Several literature reports suggest that such energy-saving and enhancing mechanisms may also apply with regard to human locomotion (Fukunaga et al., 2001; Hof et al., 1983; Lichtwark et al., 2007; Lichtwark and Wilson, 2007, 2006). The findings indicated a decrease in muscle fibre shortening and shortening velocity during walking and running due to tendon compliance, suggesting that the shortening velocity was reduced towards an optimal velocity in regard to maximal power output of the muscle and muscle efficiency (Lichtwark et al., 2007; Lichtwark and Wilson, 2007, 2006).

1.2.4 Tendon mechanical properties and locomotor performance

As shown in the previous chapter, the non-rigidity of tendons can facilitate the force potential of the attached muscle. The specific mechanical properties of tendons and involved muscles, the movement intensity, task and training status, however, may considerably affect the functional interplay of muscle and tendon (Ishikawa and Komi, 2008).

Recent investigations on running economy reported that more economical runners possessed a greater compliance of the quadriceps muscle tendon-aponeurosis at low levels of tendon forces compared to less economical runners (fig. 1.4A) (Arampatzis et al., 2006). It was suggested that the greater compliance allowed for an advantage in muscle force potential due to a decrease in shortening velocity of the muscle fibres, which in turn is associated with a decrease of active muscle volume at a given force and, thus, less energy costs (see also previous chapter). Furthermore, a greater tendon-aponeurosis elongation during the first phase of the step cycle may be related to an enhancement of elastic strain energy storage and return in the propulsion phase, contributing to running economy (Albracht and Arampatzis, 2006; Roberts et al., 1998, 1997). In contrast, the triceps surae muscle-tendon unit of the more economical runners showed a greater tendon stiffness and contractile strength compared to the less economical athletes, which, following the aforementioned argumentation, would indicate a potential disadvantage in regard to the muscle shortening velocity and energy storage of the tendon (Arampatzis et al., 2006). However, a simulation of the triceps muscle-tendon interaction revealed, that in low muscle activation levels - i.e. during running - the greater peak forces compensated the disadvantages of higher tendon-aponeurosis stiffness, resulting in the economic lower shorting velocity behavior of the muscle fibres described above (Albracht and Arampatzis, 2006). The authors concluded, that the functionality of the muscle tendon unit is dependent on the interaction of tendon-aponeurosis stiffness and maximal strength. In accordance, Fletcher et al. (2010) reported a high significant negative correlation of exercise intervention-induced changes of Achilles tendon-aponeurosis stiffness and energy costs of r=-0.723, thus, providing further evidence for the inverse relation of Achilles tendon-aponeurosis stiffness and human locomotion economy. Fletcher at al. (2010) argued that during running, where a substantial pre-stretch of Achilles tendon-apononeurosis does not occur (Lichtwark et al., 2007), a more compliant tendon would not provide a beneficial energy storage but rather require a greater muscle fiber shorting and/or shortening velocity, indicating a higher energy cost for the necessary muscle contraction (Alexander, 2000; Fletcher et al., 2013). In turn, a stiffer tendon may be favorable for a direct transmission of muscle forces and, thus, torque generation around the joint, reducing muscle fibre shortening and, therefore, facilitating power generation and movement economy (Biewener and Roberts, 2000; Fletcher et al., 2010; Lichtwark and Wilson, 2007). Just recently, Albracht and Arampatzis (2013) reported that following a high resistance exercise intervention on the triceps surae muscle-tendon unit of recreational runners, the increased muscle force and tendon-aponeurosis stiffness were accompanied by an enhanced running economy. However, the intervention-induced increase in Achilles tendon-aponeurosis stiffness did not lead to less tendon elongation during the stance phase and, thus, a reduction of the shortening velocity of the gastrocnemius muscle fibres. Instead, the unchanged elongation - despite the higher stiffness - was indicative of a greater tendon force application during stance phase, suggesting a greater strain energy storage and return by the tendon, which may have contributed to the improved running economy (Albracht and Arampatzis, 2013). Therefore, the functional interaction of muscle and tendon during locomotion is determined by the tendon mechanical properties and muscle strength.

Furthermore, studies on sprint athletes found a comparable relationship between knee extensor tendon-aponeurosis properties and performance level. Compared to a group of slower sprinters (Stafilidis and Arampatzis, 2007) or matched controls (Kubo et al., 2000b, 2011), athletes presented a greater elongation of the vastus lateralis tendon-aponeurosis at a given tendon force and during maximum voluntary knee extension contractions. The maximum elongation of the vastus lateralis tendon-aponeurosis was further significantly correlated with the 100 meter sprint times by $r=-0.567$ (Stafilidis and Arampatzis, 2007) and $r=-0.757$ (Kubo et al., 2000b), respectively. The potential advantage of a greater compliance of vastus lateralis tendon-aponeurosis for sprint performance is most-likely related to the aforementioned effects due to the muscle fibre force-velocity (i.e. associated decrease of shortening velocity) and force-length relationship (right shift in the direction of the plateau region, see also fig. 1.3 and chapter 1.2.3) that facilitate the muscle force potential, as well as the energy storage and return (Kubo et al., 2000b, 2011; Stafilidis and Arampatzis, 2007). However, such a relationship of tendon-aponeurosis compliance and sprint performance seems not to account for the triceps surae muscle-tendon unit (fig. 1.4B) (Arampatzis et al., 2007b; Kubo et al., 2000b, 2011; Stafilidis and Arampatzis, 2007). In comparison to endurance runners and subjects not involved in sports, sprint athletes demonstrated significantly higher stiffness (i.e. less compliance) of the triceps surae tendon and aponeurosis and greater maximum plantar flexion moments (fig. 1.4B) (Arampatzis et al., 2007b). These findings indicate an adaptation of the muscle-tendon unit to meet the requirements necessary for high sprinting performance. Greater triceps surae muscle forces produce greater plantar flexion moments at the ankle joint accompanied with an increased rate of force development. A greater tendon-aponeurosis stiffness can reduce the shortening and the shortening velocity of the muscle fibres during the concentric contraction, increasing the muscle-force potential and, in this way, the transmission of the muscle force to the bone may be improved. However, the tendon-aponeurosis properties cannot explain the performance level of sprinting or running alone and other factors may be important (Albracht and Arampatzis, 2013; Stafilidis and Arampatzis, 2007).

In contrast to the locomotion studies review above, the stiffness of the vastus lateralis tendon-aponeurosis was significantly positively correlated with performance during movement tasks featuring high muscle power output, i.e. squat and counter movement jumps as well as isometric knee extensor rate of torque development tests (r=0.64, r=0.55 and r=0.55, respectively) and accounted for up to 30% of the variance in the rate of torque development (Bojsen-Møller et al., 2005). It was suggested that the positive correlation of tendon mechanical properties and performance level of the high-force isometric and dynamic movement actions is based on the faster muscle force transmission to the bone associated with greater tendon stiffness (Bojsen-Møller et al., 2005). In this regard, it is not surprising that the age-related decrease of triceps surae and quadriceps femoris muscle strength as well as quadriceps femoris tendon-aponeurosis stiffness partly account for the impaired dynamic stability control of older adults compared to younger ones after simulated forward falls (i.e. slower and inadequate increase of the base of support during the recovery step in the elderly) (Karamanidis et al., 2008).

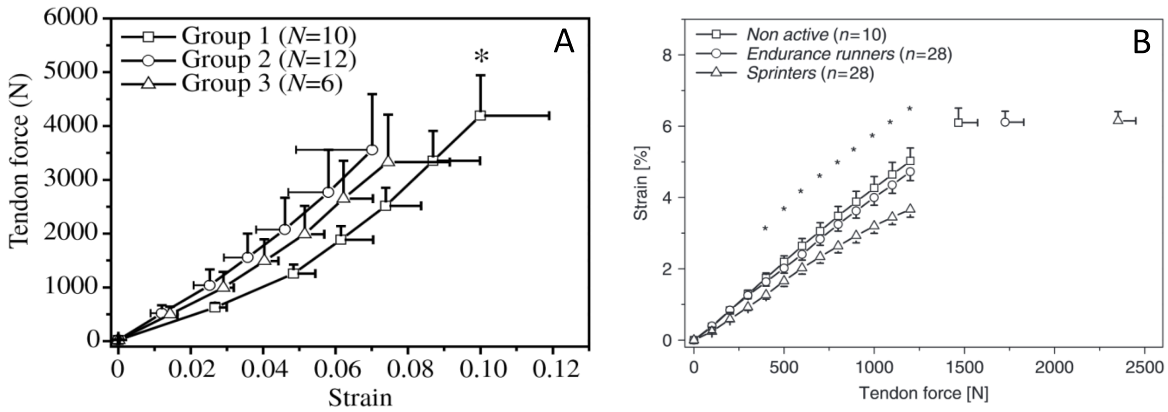

Fig. 1.4 Mechanical tendon-aponeurosis properties in relation to (A) running economy and (B) athletic activity level, respectively.
A: Tendon force-tendon strain relationship (mean±SD) of the m. quadriceps femoris tendon-aponeurosis of high economical (group 1), moderate economical (group 2) and low economical runners (group 3) during maximum knee extension effort, *: Statistically significant differences in maximal tendon strain between group 1 and the other two groups (Arampatzis et al. 2006, J. Exp. Biol., 209:3345-3357, p.3352; with permission by Company of Biologists)
B: Tendon strain-tendon force relationship (mean±SEM) of the musculus triceps surae tendon-aponeurosis during maximum plantar flexions, *: Statistically significant differences between sprinters and the other two groups. (Arampatzis et al. 2007b, J. Biomech., 40:1946-1952, p.1949; with permission by Elsevier)

Furthermore, tendon mechanical properties may contribute to the prevention of tendon injuries. A greater tendon stiffness would allow physiological levels of strain to maintained at greater muscle forces, which could be important for tendon strain injuries in particular. Strain was shown to be the primary mechanical parameter governing tendon damage accumulation and injury (Wren et al., 2003). The relationship of the actual tendon strain during loading to the ultimate strain refers to the “safety factor”, indicating a greater safety factor with a stiffer tendon

(Ker et al., 1988). Therefore, the improvement of tendon properties by specific exercises may be crucial to reduce the risk of tendon strain injuries. However, to the best of my knowledge, no study, to date, has systematically investigated the effect of improved tendon properties in vivo on sport-related tendon injury prevalence. Nevertheless, Fredberg et al. (2008) showed that eccentric training and stretching significantly reduced the frequency of ultrasonographic abnormalities in the patellar tendons of professional soccer players (Fredberg et al., 2008). Kraemer and Knobloch (2009) reported a significant reduction of Achilles and patellar tendinopathy following a proprioceptive balance training intervention for 3 years on female elite soccer players with a significant dose-response relationship (Kraemer and Knobloch, 2009). Although these studies did not measure tendon properties, the results indicate the benefits of prevention training on the reduction of the risk of tendon injuries. Besides tendon injury prevention, tendon mechanical properties are a focus of treatment in tendon injury therapy. In the course of pathologies like rupture or tendinopathy, tendon properties have been reported to be impaired (Arya and Kulig, 2010; Helland et al., 2013). Using specific therapeutic exercises, however, the tendon properties and function could at least partly be restored (Kongsgaard et al., 2009; Larsson et al., 2012; Malliaras et al., 2013a; Stergioulas et al., 2008), giving further importance to the controlled loading of tendons to improve tendon properties.

Taken together, current research provides evidence for the importance of the tendon mechanical properties for locomotion and movement performance. During stretch-shortening exercises, more compliant tendons may enhance the muscle performance due to the force-length-velocity relationship of the muscle fibres as well as the storage and return of elastic strain energy. In certain movements, where a substantial pre-stretch of the tendon does not occur, a stiffer tendon may be beneficial for working muscle fibres since muscle shortening is reduced compared to compliant tendons, allowing for a faster force generation and power production. The aforementioned interaction of muscle and tendon affects locomotion economy, as well as the muscle force potential and transmission. Furthermore, enhanced tendon mechanical properties may contribute to tendon strain injury prevention. On the other hand, the properties of tendons are impaired following pathologies like tendinopathy or rupture and need to be adequately restored in a therapeutic treatment. Therefore, it is essential to understand the mechanisms of tendon adaptation, not only to modulate the tendon mechanical properties by means of controlled exercise interventions (that aim to improve the muscle-tendon interaction and, therefore, locomotion performance), but also when addressing the fields of tendon injury prevention and therapy.

1.3 Tendon plasticity

As outlined in the previous chapter, the properties of tendons contribute to daily locomotion and significantly affect athletic performances. Moreover, tendons feature a remarkable plasticity, which allows them to respond to increased mechanical loading. The next chapter outlines the possibilities of the loading-induced tendon adaptation and explains the underlying mechanobiology. Subsequently, a brief literature review is given about chronic mechanical loading and tendon adaption, revealing some open topics of recent research.

1.3.1 Loading-induced tendon adaptation

Tendons are sensitive to their mechanical environment and chronic loading affects their mechanical and morphological properties. Indications for tendon plasticity emerged from cross-sectional studies which found different tendon mechanical and morphological properties between study participants in different groups according to their physical activity levels (Kallinen and Suominen, 1994; Kongsgaard et al., 2005; Kubo et al., 2000a, 2000b; Magnusson and Kjaer, 2003; Rosager et al., 2002). For example, Rosager et al. (2002) and Magnusson and Kjaer (2003) reported greater Achilles tendon cross-sectional areas in runners compared to non-runners, indicating a functional adaption to the exposure of repetitive loading. Further, Arampatzis et al. (2007b) found a higher Achilles tendon-aponeurosis stiffness and maximum triceps sure muscle strength in sprinters compared to endurance runners and subjects not active in sports (fig. 1.4B), which demonstrated an adaptation of the Achilles tendon in an intensity-dependent manner.

To date, it is well evidenced that tendons adapt to loading by changing their mechanical (i.e. stiffness), material (i.e. Young's modulus) and morphological (i.e. cross-sectional area and tendon rest length) properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Kjaer, 2004; Lavagnino and Arnoczky, 2005; Wang, 2006). The load of a tendon in terms of strain is directly related to the generated force of the attached muscle. Increases in muscle strength are accompanied by an increase of tendon stiffness (Arampatzis et al., 2007b; Muraoka et al., 2005), to maintain physiological ranges of strain, since the ultimate tendon strain is more or less constant (Abrahams, 1967; LaCroix et al., 2013; Loitz et al., 1989). The loading-increased stiffness is based on either a) changes of the tendon material properties (i.e. increases in Young's modulus) and/or b) changes of the tendon morphological properties (i.e. tendon hypertrophy) (Arampatzis et al., 2010, 2007a; Kongsgaard et al., 2007; Seynnes et al., 2009). While the former was suggested to bevan early mechanism to increase stiffness, the latter was considered as a long-term effect of mechanical loading (Heinemeier and Kjaer, 2011; Kjaer et al., 2009). However, no consensus exists to date regarding the relative contributions of alterations of the tendon cross-sectional area versus changes in the Young's modulus to the increase in stiffness (Heinemeier and Kjaer, 2011). Both, tendon material and morphological changes base on an elevated collagen synthesis, but also on changes of the collagen fibril morphology and levels of collagen molecular cross-linking (Heinemeier and Kjaer, 2011; Kjaer et al., 2009; Miller et al., 2005). More recently, Heinemeier et al. (2013) reported that a large degree of tendon adaptive responses may happen in the outer region of the tendon, whereas the tendon core is formed during growth and, thereafter, not subjected to considerable tissue turnover (Heinemeier et al., 2013). However, whereas several studies reported a hypertrophy of the tendon following a period of enhanced loading (Arampatzis et al., 2007a; Kongsgaard et al., 2007; Seynnes et al., 2009), no such reports exist for an exercise-induced change of tendon rest length, which hence can be excluded from being a relevant adaptive mechanism in response to mechanical loading.

Although, increases in muscle strength are accompanied by an increase in tendon stiffness to avoid non physiological levels of strain (Arampatzis et al., 2007b; Muraoka et al., 2005), the time course of adaptations are different between muscle and tendinous tissue, i.e. slower adaptation rate of the tendon (Boer et al., 2007; Kjaer et al., 2009; Kubo et al., 2012, 2010). Delayed responses on the transcriptional level of growth factors (see next chapter) of the tendinous tissue compared to the muscle most likely account for the dissimilar adaptation rates (Heinemeier and Kjaer, 2011). Furthermore, the mechanical stimulus that facilitates tissue adaptation may be different for tendon and muscle (Arampatzis et al., 2010, 2007a). Taken together, these reports indicate that potential imbalances of muscle and tendon adaptation to increased mechanical loading can occur within the time course of physical training, resulting in episodes of high tendon strain and stress (Mersmann et al., 2014). This may be of special relevance, since high patellar tendon stress has recently been shown to play a major role for the aetiology of patellar tendinopathy (Couppe et al., 2013; Kongsgaard et al., 2009).

1.3.2 Mechanobiology of tendons

The reason for the adaptive potential of tendons is the receptivity of the tendon cells to external mechanical load application (i.e. strain) (Wang, 2006). The tendon cells respond to mechanical loading by changing gene expressions, protein synthesis and cell phenotype (i.e. adopt towards a synthetic phenotype) and initial responses may proceed and induce long-term tendon structure modifications, which lead to changes in the tendon’s mechanical, material and morphological properties (Wang, 2006).

When the attached muscle contracts, the load, in terms of external strain, is transmitted through the extracellular matrix on the cytoskeleton of the tendon cells via membrane attachment proteins (integrins), other transmembrane proteins (G-protein, receptor and protein kinases) and stretching-activated ion channels (fig. 1.5) (Wang, 2006; Wang and Thampatty, 2006). The deformation of the cells initiates the expression of genes and growth factors responsible for catabolic and/or anabolic cellular and molecular responses (e.g. synthesis of collagen and matrix proteins) (fig. 1.5), affecting the material and morphological tendon properties (Bosch et al., 2002; Galloway et al., 2013; Heinemeier and Kjaer, 2011; Kjaer, 2004; Lavagnino and Arnoczky, 2005; Sullivan et al., 2009; Wang, 2006; Yang et al., 2004). In particular, the stimulation of the collagen synthesis and procollagen expression seems to be mediated by growth factors (fig. 1.5) like insulin-like growth factor 1 (IGF-I), transforming growth factor-ß-1 (TGF-ß-1) plus its binding proteins and interleukin-6, whose interstitial concentration was shown to be increased following acute exercise (Heinemeier et al., 2012; Kjaer et al., 2009; Wang, 2006). Consequently, collagen synthesis was found to be enhanced from around 1% at rest to 2-3% after exercise and collagen synthesis rates remained elevated for at least 3 days after acute exercise (Miller et al., 2005). Accordingly, a single bout as well as long-term loading induced elevated collagen synthesis responses (Langberg et al., 2001, 1999; Miller et al., 2005). The loading-induced elevation of collagen synthesis rates seems well evidenced by means of different measurement approaches of in vivo tendon collagen synthesis (e.g. microdialysis, tendon biopsies, analysis of mRNA expression) (Heinemeier and Kjaer, 2011). Furthermore, the application of static stress and dynamic strain has been shown to inhibit interstitial collagenase mRNA expression in relation to the strain amplitude, indicating an inhibition of catabolic cell responses following loading (Arnoczky et al., 2008, 2004; Lavagnino et al., 2008, 2003). In addition to the collagen content, the degree of cross-links between existing collagen molecules (especially enzymatic derived cross-links via lysyl oxidase) seems likely to be stimulated by loading. Enhanced levels of lysyl oxidase expression were reported following loading, indicating that an increasing degree of cross-links could be part of the tendinous tissue response (Avery and Bailey, 2005). More recently, tendon stem cells were described as a new group of tendons cells and it seems that these cell type may also account for loading-induced cell proliferation as well as collagen synthesis and, thus, adaptive responses (Bi et al., 2007; Wang et al., 2012; Yin et al., 2010).

The transmission of external strain via the extracellular matrix to the tendon cells was suggested by two modes: a) cell deformation and b) fluid flow induced shear stress (Lavagnino et al., 2003). With increasing strain, a loss of collagen crimp and an increase in fibre recruitment was observed (Hansen et al., 2002; Schatzmann et al., 1998), which very likely results in an increased number of cells being deformed (Arnoczky et al., 2002a) inducing adaptive processes in an intensity-depended manner (Arnoczky et al., 2004; Lavagnino et al., 2008, 2003). Beside cell deformation, fluid flow-induced shear stress seems to be a another mechanism of external strain transmission to the tendon cells that affects cellular responses (Archambault et al., 2002; Giori et al., 1993; Lavagnino et al., 2008). Lavagnino et al. (2008) reported that fluid flow-induced shear stress is mediated by the strain rate and found reduced catabolic cell responses with increased strain rate.

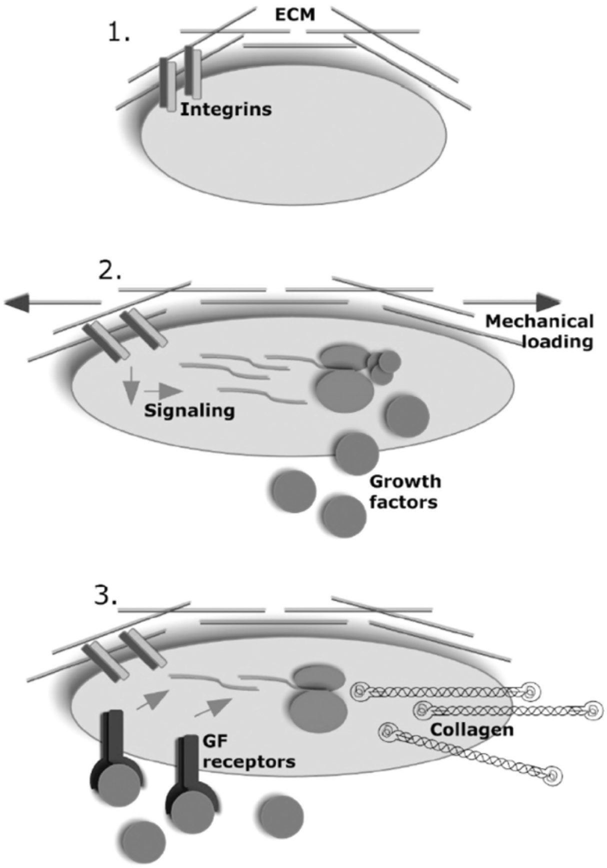

Fig. 1.5 Schematic illustration of a possible mechanism for loading-induced collagen synthesis.
1. Tendon cell is connected to the extracellular matrix (ECM) via membrane proteins (e.g. integrins); 2. Mechanical load induces transcription and synthesis of growth factors (GF) via altered intracellular signaling; 3. Autocrine/paracrine action of growth factors leading to increased collagen transcription and synthesis. (Heinemeier and Kjaer 2011, J. Musculoskelet. Neuronal. Interact., 11:115-123, p.117; with permission by the author)

Moreover, the tendon mechanical and morphological properties seem to be affected by the factor gender, with lower stiffness and Young's modulus values reported for the tendon-aponeurosis of women compared to men (Kubo et al., 2003a). Furthermore, men seem to show greater increases in collagen synthesis following exercising compared to women (Magnusson et al., 2007; Miller et al., 2007; Sullivan et al., 2009). It was suggested that estrogen levels directly and indirectly (via associated low levels of relevant growth factors) compromise the collagen synthesis in females (Kjaer et al., 2009; Magnusson et al., 2007). The role of gender specificity in the time-course of long-term adaptation of tendons to mechanical loading has, to the best of my knowledge, not yet been fully investigated.

Further, the mechanotransduction mechanisms, by which cells sense mechanical loading and translate them into the biochemical signals (fig. 1.5) that induce tissue adaptive responses, are still not completely understood (Heinemeier and Kjaer, 2011; Wang, 2006). It is also still unknown, to what extent an increased collagen synthesis contributes to either tendon hypertrophy or an alteration of the tendon material properties (Kjaer et al., 2009).

1.3.3 Tendon adaptation to chronic mechanical loading in vivo

Against earlier assumptions, research over the past two decades provided profound evidence that tendinous tissue is highly metabolically active and responds to mechanical loading (Arnoczky et al., 2002a; Langberg et al., 2001; Lavagnino and Arnoczky, 2005). An elevated collagen synthesis rate seems to play the key role for adaptive mechanisms like collagen content, changes of fibril morphology or cross-linking of molecules that affect the tendon material and morphological properties (Heinemeier and Kjaer, 2011; Miller et al., 2005). Cellular and molecular adaptive responses are triggered by mechanical loading (Wang, 2006). Therefore, mechanical loading in terms of strain is an important regulator for tendon adaptation.

To date, the adaptive responses of human tendons in vivo to mechanical loading are well documented by many longitudinal exercise intervention studies (Arampatzis et al., 2010; Carroll et al., 2011; Foure et al., 2011, 2010; Hansen et al., 2003; Houghton et al., 2013, 2013; Kongsgaard et al., 2007; Kubo et al., 2010, 2006, 2002; Malliaras et al., 2013b; Seynnes et al., 2009). In 2001, Kubo and colleagues were the first to report an increase in patellar tendon stiffness and Young's modulus following twelve weeks of exercise-based loading (Kubo et al., 2001). A training intervention-induced region specific hypertrophy of the patellar and Achilles tendon were initially reported by Kongsgaard et al. (2007) and Arampatzis et al. (2007a), respectively.

Although most intervention studies demonstrated changes of the tendon properties following the training, the reported adaptive responses were notably different between studies. Comparing the different publications, it can be concluded that the extent of the adaptation maybe related to the applied loading condition (e.g. intensity, duration, repetitions, sets, intervention duration and training frequency per week). For example, the intervention studies of Arampatzis et al. (2010, 2007a), Kongsgaard et al. (2007) and Malliaras et al. (2013a) reported a considerable effect of the loading intensity on tendon adaptation, with increases in stiffness solely observed using higher intensities. The few intervention studies applying a plyometric training reported controversial findings of intervention-induced tendon stiffness increases (Foure et al., 2010a, 2010b, 2011) or decreases (Houghton et al., 2013), most likely due to the different jumping exercise conditions, loading intensities and intervention durations. Furthermore, the exercise intervention of Albracht and Arampatzis (2013) on the Achilles tendon of runners induced an increase in stiffness accompanied by an improvement of running economy, while Fletcher et al. (2010) did not find significant improvements in tendon properties and running economy using a comparable training regimen. The shorter intervention duration and lower loading intensity in the latter study may be responsible for the lack of significant adaptation. However, the study reported a significant correlation of intervention-associated changes in stiffness and running economy of r=-0.723, indicating a strong relation of tendon properties and energy cost of running (Fletcher et al., 2010). Taken together, the loading conditions of the applied intervention protocols were set to different levels, using high and low intensities, short and long durations of the single load and different numbers of repetitions and sets. The variety of the conditions used and associated adaptive responses indicate that tendon adaptation strongly depends on the applied loading conditions.

However, though a few review articles were published on that topic (Arampatzis et al., 2009; Heinemeier and Kjaer, 2011; Magnusson et al., 2008; Magnusson et al., 2003), no systematic review or meta-analysis that aimed to analyse the specific loading conditions with respect to the associated adaptive changes of the tendon's mechanical, material and morphological properties in humans has been conducted thus far. Therefore, our understanding of human tendon plasticity in vivo is still deficient. Such meta-analysis could provide valuable information about the effectiveness of certain loading conditions for tendon adaptation, and, thus, the improvement of tendon properties.

Furthermore, there is only little information from single systematic longitudinal studies about the effect of controlled modulations of specific parameters of the mechanical strain stimulus on human tendon adaptation. As described in the previous chapter, the transfer of the external strain on the cellular level initiates the expression of genes responsible for catabolic and/or anabolic cellular and molecular responses (e.g. collagen synthesis), which affect the tendon properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Kjaer, 2004; Lavagnino and Arnoczky, 2005; Wang, 2006). From a mechanobiological point of view, four main parameters of the applied mechanical load (i.e. strain) may affect the adaptive response of tendons: magnitude, frequency, rate and duration (Arnoczky et al., 2002a; Lavagnino et al., 2008; Yamamoto et al., 2005, 2003; Yang et al., 2004). Recent experiments on the human Achilles tendon investigated the effect of strain magnitude and strain frequency on the tendon properties in vivo by means of controlled exercise interventions (Arampatzis et al., 2010, 2007a). In a first intervention, the participants exercised at a low strain magnitude (2.5-3%) on one leg and a high strain magnitude (4.5-5%) on the other leg with the same strain frequency and loading volume (Arampatzis et al., 2007a). The target strain magnitudes were applied by means of isometric plantar flexions featuring intensities of 55% (i.e. low strain magnitude) and 90% (i.e. high strain magnitude) of the maximum voluntary contraction, respectively. After 14 weeks of exercising, a significant increase of the Achilles tendon stiffness and Young's modulus (36% and 23%, respectively) as well as a region specific tendon hypertrophy, was only found in the leg trained by the high strain magnitude protocol (fig. 1.6) (Arampatzis et al., 2007a). The findings provided further evidence for a threshold of strain magnitude, which must be exceeded to induce adaptive responses of the tendinous tissue. Since the low strain magnitude protocol did not significantly affect the mechanical and morphological tendon properties, the stimulus was considered as insufficient to superimpose the habitual loading and induce a homeostatic perturbation. The finding was supported by further intervention studies, which only reported a significant increase of tendon stiffness following a training using high contraction intensities, i.e. 70% one repetition maximum (RM) (Kongsgaard et al., 2007) and 80% eccentric RM (Malliaras et al., 2013b), respectively. Taken together, strain magnitude may play a key role for tendon adaptation.

In regard to the effect of a modulation of the strain frequency, Arampatzis et al. (2010) conducted a second experiment using the same approach (i.e. low and high strain magnitude), but at higher strain frequency (0.5 Hz, 1s loading, 1 s relaxation), allowing for a direct comparison with the previous study (0.17 Hz, 3 s loading, 3 s relaxation). In accordance to the earlier study, only the high strain magnitude protocol induced changes of the tendon mechanical properties (fig. 1.6). When comparing the effectiveness of the two high strain magnitude protocols at the different frequencies (0.17 versus 0.5 Hz), pronounced adaptive responses regarding the mechanical and morphological properties were observed in the low strain frequency intervention (fig. 1.6). The authors suggested that the time of the single load application (i.e. strain duration) during the high strain frequency protocol (i.e. 1 s) was maybe to low for an appropriate transmission of the external strain to the cellular level to stimulate cellular adaptive responses compared to the longer time during the low strain frequency protocol (i.e. 3 s). It was concluded that, due to the time-dependent properties of tendon tissue, a high strain magnitude applied at a low strain frequency is more effective compared to a higher frequency.

However, the effect of strain rate and strain duration on the adaptive responses of human tendons has not been investigated thus far. Knowledge about the effect of the two parameters (i.e. rate and duration) of mechanical strain application would deepen the fundamental understanding of tendon adaptation and could contribute to the development of strategies for the improvement of human locomotor performance, as well as tendon injury prevention and rehabilitation.

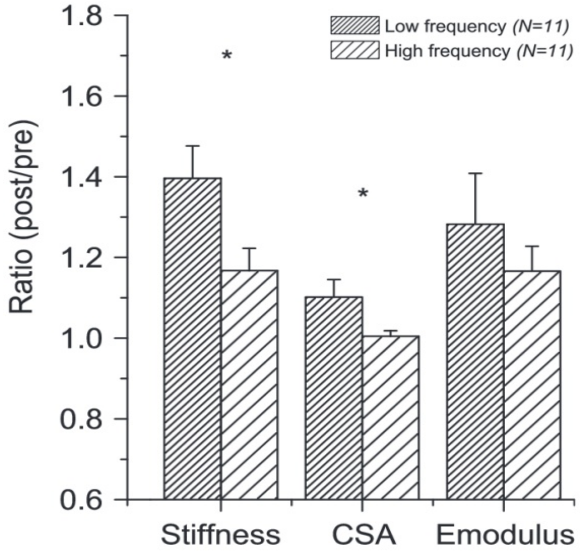

Fig. 1.6 Intervention-induced adaptations of the Achilles tendon related to the strain frequency.
Displayed is the ratio (post- to pre-exercise values) of the stiffness of the musculus triceps surae tendon-aponeurosis (Stiffness), average cross-sectional area (CSA) and elastic modulus (Emodulus) of the Achilles tendon for the low strain frequency (0.17 Hz; 3 s loading, 3 s relaxation) and high strain frequency(0.5 Hz; 1 s loading, 1 s relaxation) exercise protocols (14 weeks, 4x/week) that were both conducted using a high strain magnitude. *: Statistically significant differences between low- and high-frequency exercise protocols. (Modified from Arampatzis et al. 2010, J. Biom., 43:3073-3079, p.3077; with permission by Elsevier)

As shown, tendons feature a remarkable plasticity in response to mechanical loading and, therefore, tendon properties may determined by habitual loading pattern (Couppe et al., 2008; Kongsgaard et al., 2005; Kubo et al., 2000b; Magnusson and Kjaer, 2003; Rosager et al., 2002). In consequence, differences in daily loading of two extremities would lead to notable side-to-side differences. Only a few investigations reported differences between tendon properties of both legs in humans participating in a sport that features a side-related loading profile (Couppe et al., 2013, 2008). To my knowledge, no study investigated the effect of side-dependent daily loading profiles (i.e. foot/leg dominance) of both legs on the tendon properties in a normally active population (no side-specific sportive or professional mechanical loading). Foot preference is a well-known phenomenon and is associated with the respective predominant loading of either the left or right leg (Peters, 1988; Valderrabano et al., 2007; Wang and Watanabe, 2012), which could affect the tendon properties significantly. From a methodological point of view, laterality of tendon properties is an important issue in the field of tendon adaptation research, because side-symmetry is commonly assumed. For example, in almost all cross-sectional studies the tendon properties have been investigated on one leg as a representative for both sides (Rosager et al., 2002; Kongsgaard et al., 2005; Stenroth et al., 2012). Furthermore, clinical studies examined the therapeutic treatment following Achilles tendon rupture or tendinopathy with respect to the healthy side, assuming similar tendon properties of both legs in a healthy state (Silbernagel et al., 2006; Couppe et al., 2013; McNair et al., 2013). However, side-symmetry has not been given evidence yet. An investigation that compares the tendon properties between both legs could clarify potential laterality effects and draw conclusions in regard to methodological study designs for the assessment of tendon properties and adaptation.

In conclusion, although numerous studies evidenced tendon plasticity in response to mechanical loading, the importance of several single loading factors and their interaction for tendon adaptation is not completely understood. A systematic review and meta-analysis that compares recent intervention studies in regard to the applied factors and the respective adaptive responses would contribute to a better understanding of tendon plasticity. In addition to a comparative analysis, a study-based approach by means of a controlled modulation of - to date experimentally neglected - loading factors (e.g. strain rate and strain duration) in separate longitudinal exercise interventions could be applied to investigate their significance. Both research approaches may provide valuable information on the characteristics of effective mechanical stimuli in regard to the improvement of tendon properties in the context of athletic performance and tendon injury prevention. From a methodological point of view, the effect of side-dependent loading profiles (i.e. foot dominance) on the tendon properties should be clarified to consider the aspect of laterality in study designs.

1.4 Methodological approaches to investigate tendon properties in vivo

The following paragraph discusses the methods for the assessment of tendon properties. Since the present thesis focuses on the adaptive changes of the mechanical, material and morphological properties of human tendons in vivo, only relevant non-invasive techniques will be presented. Furthermore, in regard to the research field of tendon plasticity, to the best of my knowledge, only the Achilles or patellar tendon were the focus of investigation and, thus, solely methodological aspects for the measurement of Achilles and patellar tendon properties are considered in what follows.

The possibility to investigate the mechanical, material and morphological properties, as well as the plasticity of tendons, in response to chronic loading in vivo emerged from the development of advanced measurement techniques in the past 20 years. In particular, the improvement of the real-time brightness-mode ultrasound technology enabled researchers to study the tendon elongation during muscle contractions (Fukashiro et al., 1995; Kubo et al., 1999). Furthermore, magnetic resonance imaging (MRI) is, to date, the 'gold standard' in determining the morphological properties of tendons (Couppé et al., 2014).

1.4.1 Measurement of tendon morphological properties

The morphological properties of a tendon (i.e. length and cross-sectional area (CSA)) can be determined by imaging techniques. MRI is the preferred assessment tool (Arampatzis et al., 2010, 2007a; Carroll et al., 2011; Hansen et al., 2003; Kongsgaard et al., 2007; Kubo et al., 2002, 2001; Magnusson et al., 2001; Magnusson and Kjaer, 2003; Seynnes et al., 2009), since the images feature high resolution and good contrast between different tissues compared with other available imaging modalities (Couppé et al., 2014). Basically, sagittal images are used to detect the borders of the tendon (i.e. origin and insertion) and every transversal slice within these borders is segmented in order to determine the CSA of the tendon (fig. 1.7).

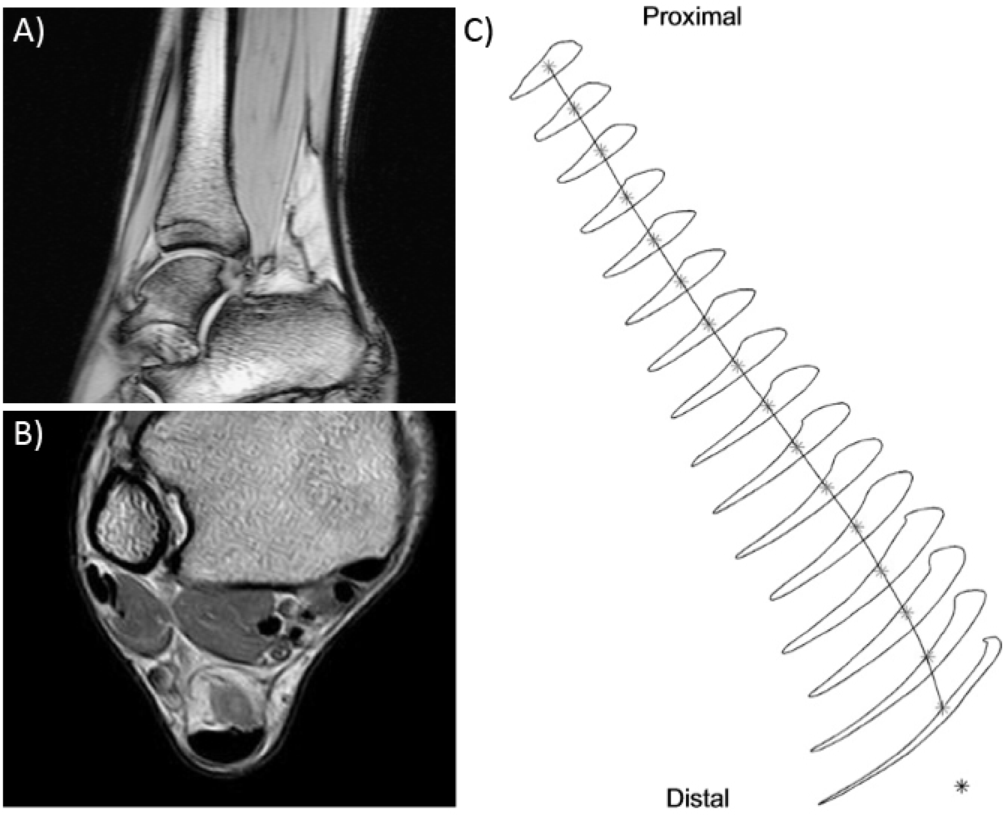

Fig. 1.7 Measurement of the tendon morphological properties (i.e. length and cross-sectional area (C)) by means of sagittal (A) and transverse (B) magnetic resonance images exemplary for the free Achilles tendon. (Arampatzis et al. 2010 J. Biom., 43:3073-3079, p.3075; with permission by Elsevier)

The tendon length can be calculated as the curved path through the centroids of the single CSAs (fig. 7) (Arampatzis et al., 2010, 2007a; Seynnes et al., 2009). To consider a region specific shape and/or adaptation of tendons following mechanical loading, not just the average CSA, but also different positions along tendon length need to be considered (fig. 7) (Arampatzis et al., 2007a; Kongsgaard et al., 2007; Seynnes et al., 2009). Although several studies used ultrasonography to determine the tendon CSA (Foure et al., 2010, 2013; Houghton et al., 2013; Maganaris, 2002; Urlando and Hawkins, 2007), the reliability of this method was reported to be poor (Ekizos et al., 2013).

1.4.2 Measurement of tendon mechanical and material properties

The calculation of the tendon stiffness is based on the tendon force-tendon elongation relationship (see also chapter 1.2.2, fig. 8C). The tendon force can be calculated from the joint moment exerted during a maximum voluntary isometric contraction (MVC) of the corresponding muscles (Achilles tendon: m. triceps surae; patellar tendon: quadriceps femoris) and recorded by a dynamometer (Arampatzis et al., 2009; Heinemeier and Kjaer, 2011; Magnusson et al., 2008). However, the moment measured by the dynamometer is additionally affected by a) the gravitational forces of the dynamometer arm-body segment system, b) the non-rigidity of the dynamometer arm-body segment system and c) inertia (Herzog, 1988; Winter et al., 1981). The effect of gravitational forces depends on the weight and position of dynamometer arm and the attached body segment (foot or shank) with respect to the axis of rotation, and have a strong effect on the measured moment (Herzog, 1988). Furthermore, during the MVC the axes of joint and dynamometer do not remain aligned due to the non-rigid construction of the dynamometer as well as soft tissue deformation. Consequently, the lever arm of the joint and dynamometer differs during the contraction, which results in a discrepancy in the measured moment by the dynamometer and the actual joint moment (Arampatzis et al., 2005a, 2005b). The effect of inertia can be ignored, since the isometric MVC is performed in a quasi static condition (Herzog, 1988). It has been shown that differences between measured (by the dynamometer) and resultant (by calculation) joint moment during the MVC account for an overestimation of the joint moment by 6-10% at the ankle joint (Arampatzis et al., 2005a) and 4-7% at the knee joint (Arampatzis et al., 2004). Therefore, it was suggested to calculate the resultant joint moment using an inverse dynamics approach to consider the effect of axes misalignment and gravity and, thus, enhance the accuracy of the joint moment assessment (Arampatzis et al., 2005a; Arampatzis et al., 2004). The measured joint moment exerted during the MVC is further affected by the contribution of the antagonistic muscles to the order of 3-8% (Mademli et al., 2004; Magnusson et al., 2001; Rosager et al., 2002). Mademli et al. (2004) developed an appropriate method to quantify the antagonistic muscle contribution. The experimenters used electromyography (EMG) to record the activity of the antagonistic muscles during the MVC. Based on the relationship between the EMG amplitude of the antagonistic muscles and the generated moments during sub-maximal isometric agonistic contractions, the corresponding antagonistic moment during the MVC could be calculated and considered in the assessment of the net moment (Mademli et al., 2004). In regard to the ankle joint moment, several additional muscles contribute to the plantar flexion moment (e.g. m. tibialis posterior, m. flexor halucis longus, m. flexor digitorum longus). To the best of my knowledge, no methods exist, to date, to measure their contribution to the actual plantar flexion moment. However, these muscles feature comparably small CSAs and lever arms, indicating a minor effect on the generated moment. Furthermore, the joint angles (Achilles tendon: ankle and knee; patellar tendon: knee and hip), in which the MVC is performed, affect the maximum moment due to the force-length relationship of the respective muscles (Arampatzis et al., 2005a; Arampatzis et al., 2004; Cresswell et al., 1995) and, thus, need to be considered in the joint moment determination. To calculate the tendon force, the determined joint moment is divided by the tendon lever arm. Several studies (Foure et al., 2010; Kongsgaard et al., 2007; Kubo et al., 2007, 2001) estimated the tendon lever arm from the relation to anthropometric data like leg length (e.g. Visser et al., 1990). However, predictions of the lever arm from anthropometric measurements were reported to be poor, suggesting that imaging-based technologies remain necessary for accurate lever arm quantification (Tsaopoulos et al., 2007; Waugh et al., 2011). Different methods have been used to assess the Achilles and patellar tendon lever arm length under in vivo conditions. However, two methods have become popular: 1) the tendon excursion method (principle of virtual work) (An et al., 1984), and 2) the geometric method (Reuleaux graphical analysis) (Reuleaux, 1875). The former method is based on the ratio of the excursion of the myotendinous or osteotendinous junction to the corresponding angular rotation of the joint (An et al., 1984; Fath et al., 2013, 2010; Tsaopoulos et al., 2006). MRI or X-ray images from different joint angles allow us to determine the junction excursion (Achilles tendon: calcaneus notch; patellar tendon: tuberositas tibiae) in relation to the angular change of the joint (longitudinal axis of the tibia) (Maganaris, 2004; Maganaris et al., 2000; Tsaopoulos et al., 2006). Alternatively, the m. gastrocnemius medialis myotendinous junction displacement can be obtained by ultrasonography in relation to the corresponding angular excursion of the ankle joint (e.g. measured by an angular meter) and used to calculate the Achilles tendon lever arm (Fath et al., 2013, 2010; Maganaris, 2002). For the geometric method an origin or reference point (e.g. the instant centre of rotation, the tibiofemoral contact point or the anterior and posterior cruciate ligament intersection point) for the rotation of the segments is identified from imaging techniques such as MRI or X-ray (Baltzopoulos, 1995; Erskine et al., 2014; Tsaopoulos et al., 2006). The lever arm is calculated as the perpendicular distance from the tendon line of action to the reference point (Baltzopoulos, 1995; Churchill et al., 1998; Maganaris et al., 2000, 1998; Rugg et al., 1990; Tsaopoulos et al., 2006). However, changes of the tendon lever arm due to joint rotation and/or muscle contraction need to be considered in the calculation (e.g. Herzog and Read, 1993; Maganaris et al., 2000, 1998).

For the tendon elongation measurement a ramp MVC (~5-10 s gradual increase to minimize possible effects of the viscous tendon properties) is performed by the subject and the corresponding tendon elongation can be visualized by means of ultrasonography (fig. 1.8). For that purpose an ultrasound probe is aligned to the longitudinal axis of the respective tendon (Hansen et al., 2006; Kongsgaard et al., 2011; Reeves et al., 2003). A warm-up including several (5-6) MVC trials prior to the tendon elongation measurement is necessary to avoid effects of tendon preconditioning (Maganaris, 2003). The patellar tendon elongation refers to the displacement of the origin and insertion (distal pole of the patellar bone and tuberositas tibiae, respectively) that are visible in the same ultrasound image (fig. 1.8A) using advanced ultrasound probes (10 cm width) (Carroll et al., 2011; Hansen et al., 2006; Kongsgaard et al., 2007; Schulze et al., 2012). To measure the Achilles tendon-aponeurosis (fig. 1.8B) (Arampatzis et al., 2007a; Kubo et al., 2002) or m. vastus laterialis tendon-aponeurosis elongation (Bojsen-Moller et al., 2003; Kubo et al., 2003b; Kubo et al., 2006, 2001), insertion and origin can not be displayed in one image and, thus, the displacement of a reference point (e.g. muscle fascicle-tendon cross-point, myotendinous junction of the m. gastrocnemius medialis) during the MVC is used. This approach also includes the aponeurosis in the elongation measurement, which may, in part, feature different functions and properties than the free tendon (e.g. transverse strain) (Azizi et al., 2009; Azizi and Roberts, 2009; Finni et al., 2003; Magnusson et al., 2003). Furthermore, it is assumed that the insertion point stays constant during the contraction. However, due to deformations of the dynamometer and soft tissue during the MVC, the joint angle and, therefore, the position of the insertion changes, which leads to a significant overestimation of the tendon elongation in earlier studies (Bojsen-Moller et al., 2003; Magnusson et al., 2001; Muramatsu et al., 2001; Rosager et al., 2002). Arampatzis et al. (2008) reported that the change in the ankle angle during a maximum voluntary plantar flexion accounted for an overestimation of the actual tendon elongation by about 58%. The authors further proposed a passive tendon displacement correction method that significantly reduced the overestimation of the tendon-aponeurosis elongation (Arampatzis et al., 2008). Although the ultrasound-based tendon elongation determination is very attractive, it must be emphasised that this measurement only accounts for two dimensions of the structural deformation (i.e. longitudinal plane) and cannot display three dimensional deformations, which may be even more important in regard to tendon-aponeurosis elongation measurements (e.g. transverse strain of the aponeurosis) (Magnusson et al., 2008). With regard to the reliability of ultrasound-based patellar tendon elongation measurements reported by Schulze et al. (2012), the force and elongation data of five contractions should be averaged to achieve maximum reliability (≥0.95).

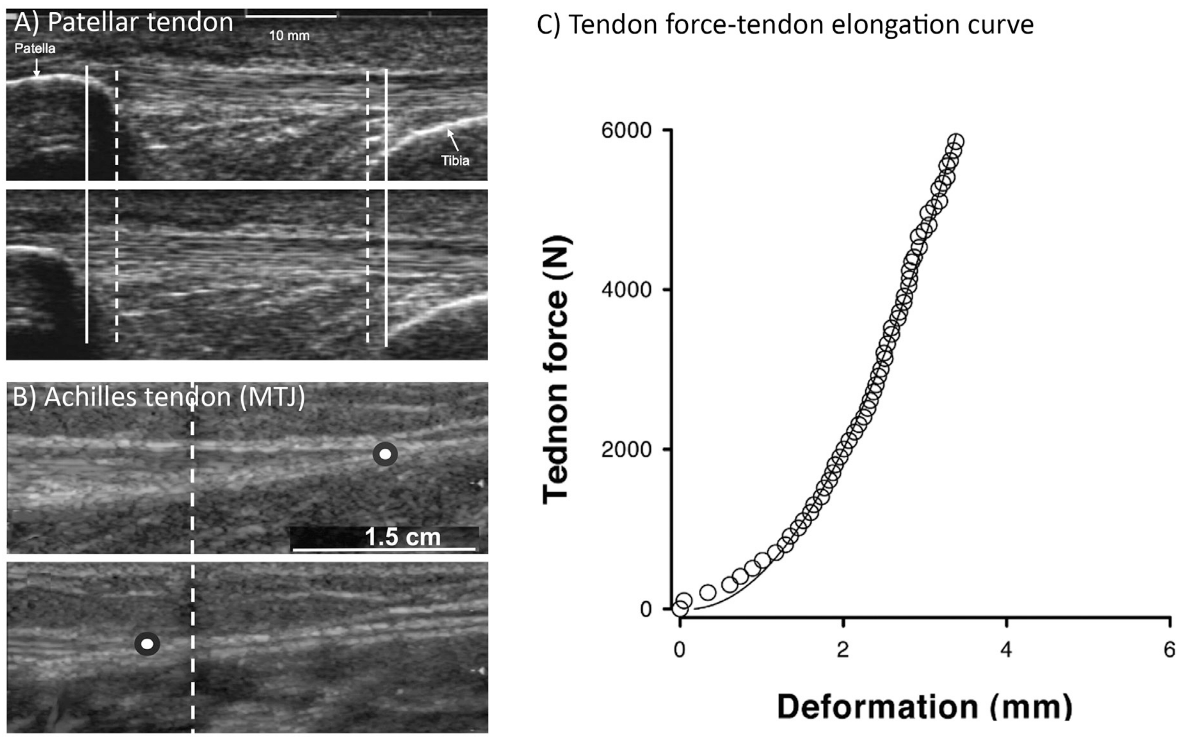

Fig. 1.8 Ultrasound images of the patellar (A) and Achilles (B) tendon during rest (upper) and ramped maximum voluntary contraction (deeper) with an exemplary corresponding tendon force-elongation curve (C). The dashed line in the patellar tendon images (A) show the patella and tibia position at rest, and the solid line the positions during a maximum voluntary contraction. The Achilles tendon elongation is indicated by the displacement of the myotendinous junction of the m. gastrocnemius medialis and Achilles tendon (MTJ, white point in upper and deeper image (B)).
(A: Carroll et al. (2011). J. Appl. Physiol., 111:508-515, p.510 (with permission by American Physiological Society); B: Morse et al. (2005) J. Appl. Physiol., 98:221-226, p.222 (with permission by American Physiological Society); C: Heinemeier and Kjaer 2011, J. Musculoskelet. Neuronal. Interact., 11:115-123, p.117(with permission by the author))

Based on the determined tendon force-elongation relationship (fig. 1.8C), and taking into consideration the aforementioned methodological aspects, the stiffness is calculated by dividing the change in tendon force by the corresponding increase of tendon elongation between 50 and 100% of the maximum tendon force or using a linear regression (Kubo et al., 2002, 2001; Schulze et al., 2012). To calculate the Young's modulus the tendon force-elongation relationship is converted to the tendon stress-strain relationship. The tendon stress is calculated as the quotient from the tendon force and the averaged CSA, and the tendon strain as the quotient from the tendon elongation and the tendon rest length (Heinemeier and Kjaer, 2011). However, the rest length measurement of the Achilles tendon needs to take into consideration the curved path of the tendon-aponeurosis (Stosic and Finni, 2011) as well as slackness (i.e. no force acting on the tendon due to dorsal flexed ankle angle) (De Monte et al., 2006). The Young's Modulus is calculated by dividing the change in tendon stress by the corresponding increase of tendon strain between 50 and 100% of maximum tendon stress, or by means of a linear regression (Arampatzis et al., 2010, 2007a).

To date, no standardized protocol for the testing of tendon mechanical, material and morphological properties exists. Differences in the calculation of tendon force (e.g. consideration of axes misalignment, gravitational forces, antagonistic muscle activation) and tendon elongation measurement (e.g. reference landmark, joint angle change) may partly explain the variation between the reported values in the literature.

2. Purpose of the thesis

From a mechanobiological point of view, the effect of the mechanical environment on the biology of the adaptive tendinous tissue determines the characteristics of the tendon properties. To understand this effect, it is necessary to investigate the mechanical conditions that may affect the tendon adaptive responses in vivo. Although numerous studies provided evidence for the influence of mechanical loading on tendon properties, there is only little systematic and ongoing research in regard to specific and controlled mechanical stimuli for tendon adaptation. Thus, the effectiveness of single independent parameters of the applied mechanical strain stimulus and their interaction, as well as the influence of more general loading conditions of an exercise intervention (e.g. intervention duration and training frequency) or unspecific daily loading profiles (e.g. foot dominance) on tendon adaptation is still not completely understood. A fundamental understanding of tendon plasticity is essential, particularly when looking at human locomotor performance as well as tendon injury prevention and rehabilitation. Therefore, the overall objective of the present thesis is to gain insight into the effect of the mechanical environment on the associated biological responses.

More precisely, the present thesis addresses some specific issues of the international literature regarding tendon plasticity:

Symmetry of Achilles tendon properties between legs is commonly assumed in clinical studies that investigate therapeutic treatments following tendon rupture or tendinopathy, using the healthy side as a reference to quantify regenerative or pathological changes of the affected leg. Furthermore, in almost all cross-sectional studies examining tendon properties in different populations, the measurements were conducted on one leg following the assumption of similar tendon properties of the left and right side. However, foot preference in daily activities (i.e. foot dominance) is a well-known phenomenon and associated with respective predominant loading of either the left or right leg, which in turn could affect the symmetry of tendon properties as well. To the best of my knowledge, there is no evidence yet to support the assumption of symmetrical tendon properties between both legs in a normally active population (i.e. no sportive or professional side-specific loading).

To date, a lot of exercise intervention studies reported the adaptive potential of tendons in response to mechanical loading. However, different levels of the applied mechanical loading conditions (e.g. intensity, duration, repetitions, sets, intervention duration and training frequency per week) affected the adaptive responses across studies, indicating that the loading conditions may determine the material and morphological adaptive responses of tendons. However, only a few studies investigated the effect of modulating single loading parameters on tendon adaptive responses systematically and, therefore, the importance of specific loading conditions for tendon adaptation is still not completely understood. A systematic comparative analysis of recent intervention studies on tendon adaptation that considers the different loading factors and the respective adaptive changes of the mechanical, material and morphological tendon properties would shed light on the effectiveness of certain loading levels for tendon adaptation, but has not been conducted thus far. Such a systematic review and meta-analysis may provide crucial information on how to facilitate tendon adaptation.

Furthermore, from a mechanobiological point of view, four parameters of the applied strain stimulus may affect the tendon adaptive responses: magnitude, frequency, rate and duration. Whereas the effects of strain magnitude and strain frequency have been recently investigated by means of controlled exercise interventions, the effects of a modulation of the strain rate and strain duration have not been studied thus far. In vitro studies suggested that strain rate-related fluid shear stress and time-dependent cell deformation are relevant factors of the mechanical strain stimuli for tendon adaptation. Therefore, high strain rates and long strain durations may facilitate human tendon adaptation in vivo. Knowledge about the effect of strain rate and duration on tendon adaptation could deepen the current understanding about the most efficient mechanics for biological adaptive responses of tendons.

With regard to the outlined deficits in the international field of tendon research, the conducted studies within the present thesis pursued the following objectives:

The purpose of the first study was to investigate the symmetry of the mechanical, material and morphological properties of the Achilles tendon of the non-dominant and dominant leg in a population with no side-specific sportive or professional mechanical loading. We hypothesized that, as a potential result of side-dependent loading during normal daily activities (i.e. foot dominance), we would find asymmetrical tendon properties between both legs.

The second study aimed to systematically review recent literature on human tendon adaptation following chronic mechanical loading in vivo and to meta-analyse the applied loading conditions and the respective adaptive responses as well as methodological aspects. The tendon mechanical, material and morphological properties will be considered to account for a complete description of the adaptive processes. It was expected that the adaptive changes of tendon properties following the intervention-induced loadings strongly depend on the different loading conditions and that certain conditions may prove to be more effective for tendon adaptation.

Finally, the purpose of the third study was to investigate the effect of a controlled modulation of strain rate and strain duration on human Achilles tendon adaptation in vivo. Based on findings of in vitro studies, we hypothesized that an increase of both strain duration or strain rate with regard to a reference stimulus would enhance the adaptive response of the tendon.

3. First study:

Asymmetry of Achilles Tendon Mechanical and Morphological Properties Between Both Legs

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3.1 Abstract

Although symmetry of Achilles tendon (AT) properties between legs is commonly assumed in research and clinical settings, different loading profiles of both legs in daily life (i.e., foot dominance) may affect the tendon properties in a side-depended manner. Therefore, AT properties were examined with regard to symmetry between legs. Thirty-six male healthy adults (28 ± 4 years), who were physically active but not involved in sports featuring dissimilar leg load participated. Mechanical and morphological AT properties of the non-dominant and dominant leg were measured by means of ultrasound, magnetic resonance imaging and dynamometry. The AT of the dominant leg featured a significant higher Young's modulus and length ($P < 0.05$) but a tendency toward lower maximum strain ($P = 0.068$) compared with the non-dominant leg. The tendon cross-sectional area and stiffness were not significantly different between sides. The absolute asymmetry index of the investigated parameters ranged from 3% to 31% indicating poor AT side symmetry. These findings provide evidence of distinct differences of AT properties between both legs in a population without any sport-specific side-depended leg loading. The observed asymmetry may be a result of different loading profiles of both legs during daily activities (i.e., foot dominance) and challenges the general assumption of symmetrical AT properties between legs.

Key words:
Laterality, tendon material properties, sidedness, footedness, tendon function, MRI.

3.2 Introduction

Tendons transmit the forces of the corresponding muscle to the skeleton and, therefore, contribute to various aspects of daily human movement performance (Kawakami et al., 2002; Arampatzis et al., 2006; Karamanidis et al., 2008; Albracht & Arampatzis, 2013). Tendinous tissue is highly sensitive to habitual mechanical loading. For example, an increase of muscle force that is regularly applied on the tendon increases the tendon's stiffness (Kubo et al., 2002; Reeves et al., 2003; Arampatzis et al., 2007b; Kongsgaard et al., 2007) in order to maintain physiological ranges of strain, as the maximum strain of tendons cannot be significantly altered (LaCroix et al., 2013). The two mechanisms that may affect tendon stiffness are: (a) changes in tendon material properties (i.e., Young's modulus) and (b) changes in tendon morphological properties (i.e., crosssectional area (CSA) and tendon rest length) (Arampatzis et al., 2007a, 2010; Kongsgaard et al., 2007; Seynnes et al., 2009).

As tendons adapt in response to mechanical loading in an intensity-depended manner (Arampatzis et al., 2007b), an asymmetrical adaptation of tendon properties to side-specific loading seems reasonable and has been demonstrated in recent studies on badminton players (Couppe et al., 2008; Couppé et al., 2013). However, most human daily activities like walking, standing, running and ascending or descending stairs require a bipedal movement pattern. Therefore, it can be argued that the daily loading profiles of left and right Achilles tendon (AT) in humans with no side-specific sportive activities are more or less similar and, in consequence, their tendons should feature symmetrical properties. In accordance to this assumption (i.e., symmetry of AT properties), a lot of clinical studies investigating therapeutic treatment following AT rupture or tendinopathy used the healthy side as a reference to quantify regenerative or pathological changes of the affected leg (Silbernagel et al., 2006; Couppé et al., 2013; McNair et al., 2013). Furthermore, in almost all cross-sectional studies examining tendon properties in different populations, the measurements were conducted on one leg following the assumption of similar tendon properties of the left and right side (Rosager et al., 2002; Kongsgaard et al., 2005; Stenroth et al., 2012).

However, foot preference in specific activities (i.e., foot dominance) is a well-known phenomenon and is associated with respective predominant loading of either the left or right leg (Peters, 1988; Valderrabano et al., 2007; Wang & Watanabe, 2012). Even gait, although it appears to be symmetrical, may show different loading profiles between the two legs (Sadeghi et al., 2001; Sadeghi, 2003; Riskowski et al., 2012). Furthermore, muscle strength can be different between the left and the right plantar flexor muscles in normal active individuals (Damholt & Termansen, 1978). Although the level of loading that triggers adaptations is higher in tendons compared with muscles (Arampatzis et al., 2007a, 2010), the above reports indicate the probability of dissimilar loading profiles between legs during daily life because of foot preference, which in turn could affect the symmetry of tendon properties as well. However, to our knowledge, the assumption of symmetrical tendon properties between both legs in a normally active population (i.e., no side-specific loading) has not been given evidence yet.

Therefore, the objective of the present study was to investigate the symmetry of the mechanical and morphological properties of the AT of the non-dominant and dominant leg in a population with no side-specific sportive or professional mechanical loading. We hypothesized that, as a potential result of side-depended loading during normal daily activities (i.e., foot dominance), we would find asymmetrical tendon properties between both legs.

3.3 Methods

3.3.1 Participants

Thirty-six male healthy adults (age: 28.3 ± 4.2 years, height: 175.6 ± 7.1 cm, weight: 76.2 ± 11.1 kg, mean ± SD) participated in the present study after giving informed consent to the experimental procedure and study approval by the university ethics committee. All participants reported no musculo-skelettal impairments of the lower limbs and were physically active (5 ± 3 h per week, 3 ± 2 times per week during the 6-month prior to the experiment). An unilateral sports activity and high-performance sports (> 12 h per week) during the past years was an exclusion criteria for the participant recruitment to avoid potential effects of asymmetrical loading pattern of both legs because of a specific activity. In order to determine the foot preference of the participants in specific daily activities (foot dominance), the approved German version (Büsch et al., 2009) of the lateral preference inventory (Chapman et al., 1987) was used and, therewith, the left and right leg were assigned to either dominant or non-dominant side, respectively.

3.3.2 Measurement of mechanical properties

To investigate the relationship of AT force to AT elongation, the subjects had to perform maximal isometric plantar flexion contractions (MVC) in seated position (i.e., hip angle 115 °) with the arms crossed in front of the chest, the knee extended, and the ankle angle at neutral position (tibia perpendicular to the sole, 0 °) on a dynamometer (Biodex-System 3, Biodex Medical Systems Inc., Shirley, New York, USA). Although the axes of the ankle joint and dynamometer were carefully aligned, dislocations of the axes during maximal contractions occur and, thus, resultant joint moments were calculated by inverse dynamics (Arampatzis et al., 2005a). Required kinematic data were provided by an infrared motion capture system (Vicon Nexus, version 1.7.1., Vicon Motion System, Oxford, UK) integrating nine cameras operating at 250 Hz. The activity of the antagonistic tibialis anterior muscle was captured using electromyography (EMG) operating at a sampling frequency of 1000 Hz (Myon m320RX, Myon AG, Baar, Switzerland) to consider its contribution to the resultant joint moment during the maximal plantar flexion contraction. The dynamometer and EMG devices were integrated via analog channels to the motion capture system (i.e., Vicon Motion System) and, therefore, directly synchronized. By examining the relationship of EMG amplitude of the tibialis anterior muscle and the generated moments while performing submaximal isometric dorsal flexion contractions, the corresponding antagonistic moment during the maximum plantar flexion could be calculated (Mademli et al., 2004). Hence, the investigated plantar flexion moments include the correction of the axes misalignment and the contribution of the antagonistic tibialis anterior muscle. The AT force was calculated by dividing the plantar flexion moment by the tendon lever arm, which was determined by applying the tendon excursion method. The method is based on the ratio of the m. gastrocnemius medialis-AT junction (MTJ) displacement to the corresponding angular excursion of the ankle joint (Fath et al., 2010). Although the tendon rigidity is required in this method, the magnitude of tendon deformation due to the ankle angle change was shown to be very low in the range used for the lever arm calculation (i.e., 5 ° dorsal flexion to 10 ° plantar flexion; De Monte et al., 2006). The alteration of the tendon lever arm because of the alignment of the tendon during the contraction was not measured for each participant but considered in the calculation of the lever arm values using the factor suggested by Maganaris et al. (1998). The AT elongation during the MVC was measured by capturing the MTJ displacement using B-mode ultrasonography. A 10 cm linear ultrasound probe (My Lab 60, Esaote Canada, Georgetown, Canada) embedded in a custom-built foam cast was smoothly fixed with Velcro straps to the shank above the MTJ, aligned in its movement direction during the contraction. A gel pad and water-based transmission gel were used to ensure acoustic coupling and improve the signal transmission. Potential relocations of the probe because of muscle deformation during the MVC were registered using an external sound-absorbing marker fixed on the skin under the field of view of the probe. The displacement of the MTJ during a ramped MVC (~ 5 s gradual increase of force) was recorded at 25 Hz and afterwards traced manually frame by frame within a custom written MATLAB interface (The Mathworks, version 2012, Natick, Massachusetts, USA). An external analog trigger signal was set manually in the beginning and the end of each trial to synchronize the data captured by the Vicon Motion System and by the ultrasound device. The trigger signal was recorded by the Vicon Motion System and caused simultaneously an optical signal in the ultrasound videos, which was tagged during the analysis of the MTJ displacement. The data series from the Vicon Motion System and the ultrasound video analysis were merged afterwards using a custom written MATLAB procedure. During the MVC, the ankle joint angle did not remain constant (Arampatzis et al., 2005b), which significantly affects the measurement of the MTJ displacement and, thus, the calculated tendon elongation (Arampatzis et al., 2008). Therefore, the MTJ displacement was recorded and analyzed using the ultrasound device in an additional trial, in which the ankle joint of the inactive subject was passively rotated by the dynamometer device over the full range of motion at 5 °/s. The limits were set at 35 ° plantar flexion and the individual maximum dorsal flexed position (~ 15 °), which was defined as the personal threshold of discomfort. The corresponding kinematic data were captured by the Vicon Motion System. The resultant function of passive MTJ displacement to ankle angle was then used to correct the displacements measured during the MVCs accordingly. To guarantee a high reliability of the tendon elongation measurement, the data of five MVC trials (at least 3 min rest between trials) were averaged according to the findings of Schulze et al. (2012).

The stiffness of the AT was calculated as the slope of the tendon force and tendon elongation ratio between 50% and 100% of the maximum tendon force by means of linear regression. In order to calculate the tendon strain (AT elongation/AT rest length), the rest length of the AT was determined. The AT rest length was measured from the tuberositas calcanei to the MTJ in 20 ° plantar flexed ankle joint and knee extension using flexible measuring tape, as in this position slackness of the inactive m. gastrocnemius medialis-Achilles tendon-aponeurosis unit has been reported (De Monte et al., 2006). The position of the tuberositas calcanei and MTJ was determined by palpating and using the ultrasound device. The maximum plantar flexion moment of the m. triceps surae was determine by performing three to five MVCs in 5 ° intervals from neutral position (i.e., 0 °) to maximum dorsal flexion angle (~ 15 °, angles measured by the dynamometer system). The maximum plantar flexion moment considered the above described corrections (i.e., axes misalignment and contribution of the antagonistic muscle) and was then used to calculate the corresponding maximum AT force.

3.3.3 Measurement of morphological properties

Magnetic resonance imaging was used to determine the morphological properties of the free AT, i.e., length and CSA along its length. Transversal and sagittal magnetic resonance imaging scans (3D HYCE (GR) sequence, TR 10 ms, TE 5 ms, flip angle 80 °, slice thickness 3 mm, 1 excitation) were captured by means of a 0.25 T magnetic resonance scanner (G-scan, Esaote). The participants lay in supine position with the hip and knee extended and the ankle fixed in relaxed position (18.5 $\pm$ 5 ° plantar flexed). The shank was aligned to the plane of the magnetic resonance scanner carefully as possible. The boarders of the free AT were detected by identifying the m. soleus-AT junction and the initial attachment on the calcaneus bone in the sagittal plane images (fig. 3.1). Every transversal slice along the free AT was segmented manually using the software OsiriX (Pixmeo SARL, version 2.5.1., Bernex, Switzerland) (fig. 3.1). The length of the free AT was calculated as the curved path through the centroids of the CSAs, which were determined by means of Delaunay triangulation (fig. 3.1). Using this method, we were able to display the three dimensional anatomical structure of the AT. To consider region specific shapes of the AT, the CSA was calculated in 10% intervals along the free AT length (Arampatzis et al., 2007a, 2010). Three independent observers analyzed the images of both legs from all subjects and the mean values of the observers were used for the statistical analysis. The Young's modulus of the AT was calculated using a linear regression of the relationship of tendon stress and tendon strain from 50% to 100% of the maximal stress. The averaged CSA of the AT along its length was taken to determine the AT stress (maximum AT force/AT CSA).

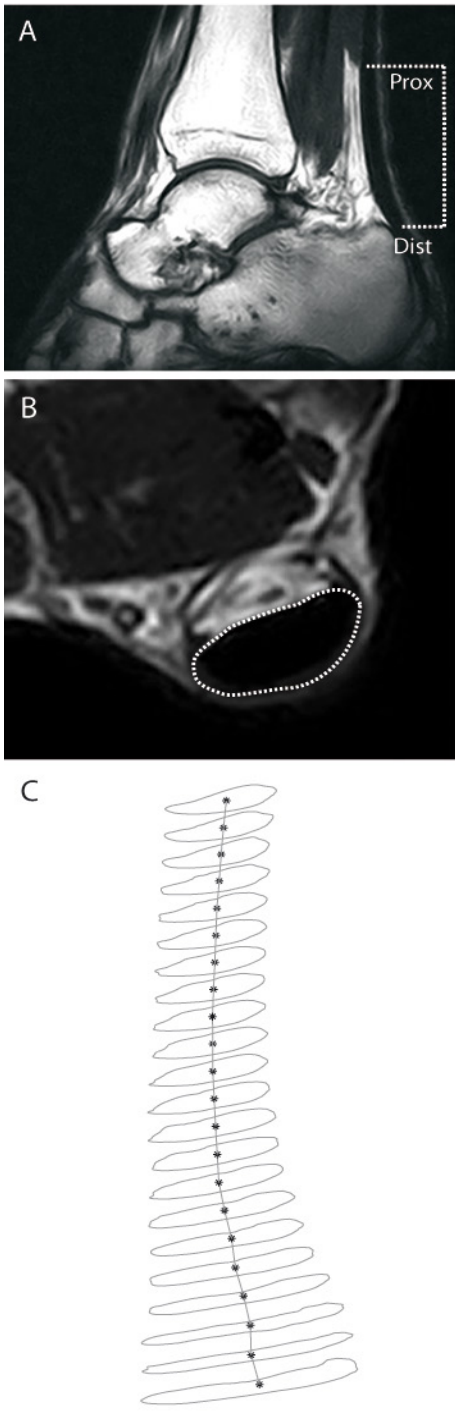

Fig. 3.1 Sagital (A) and transversal (B) magnetic resonance images of free Achilles tendon (AT) were used to investigate the morphological AT properties, i.e. length and cross-sectional area along its length (C). Transversal images served to detect the borders of free AT, the m. soleus-AT junction (Prox) and initial attachment on the calcaneus bone (Dist, A). Sagittal images within this range were segmented manually to determine the AT cross-sectional area (dotted line in B).

3.3.4 Statistics

Normal distribution of the differences of the same parameter between the non-dominant and dominant side and also for each side was checked using the Shapiro-Wilk test. If normal distribution of the side differences was given, a paired t-test was used to analyze the differences between the mechanical and morphological AT properties of the non-dominant and dominant leg. In the case of non-normal data distribution, the Wilcoxon signed-rank test was used to test for differences between sides. The Pearson correlation coefficient was calculated in order to determine the relationship of the parameters from non-dominant and dominant side, given that normal distribution of the data of each side (i.e., non-dominant and dominant) was confirmed. In the particular case that normal distribution of the data of each side could not be confirmed, the Spearman's correlation coefficient was calculated. The level of significance for all statistical procedures was set to $\alpha = 0.05$. Furthermore, to analyze the symmetry between both sides the absolute asymmetry index (AAI) was used (Karamanidis et al., 2003). AAI was calculated as:

$$AAI = \frac{|x_D - x_{ND}|}{\frac{1}{2}(x_D + x_{ND})} \times 100\% \quad \text{(Eq. 1)}$$

x_{ND} is the parameter from the non-dominant side and x_D the corresponding parameter from the dominant side. A value of AAI close to 0% indicates that the values of the non-dominant and dominant side are quite identical and, thus, symmetry is given. Finally, to investigate the agreement between the measures of the non-dominant and dominant leg, we used the Bland and Altman plots (Bland & Altman, 1986).

3.4. Results

Thirty-three of the 36 participants of the present study declared their right leg as the dominant one in the assignment to specific actions.

The maximum ankle joint moment and maximum tendon force as well as the AT lever arm were not significantly different between the non-dominant and dominant leg (tab. 3.1). In a similar manner, there was no significant difference between the sides in the maximum tendon elongation and stiffness values (tab. 3.1). Accordingly, the AT force-elongation relationship of the non-dominant and dominant leg did not presented significant differences (fig. 3.2). However, the AT rest length and length of free AT of the dominant leg were significantly greater ($P < 0.05$) and the maximum strain was lower by tendency ($P = 0.07$) compared with the non-dominant side (tab. 3.1). AT Young's modulus was significantly greater on the dominant compared with the contralateral leg ($P < 0.05$) (tab. 3.1). Neither the average CSA nor any of the 10% intervals of CSA along the free tendon length were significantly different between the AT of the non-dominant and dominant leg (tab. 3.1 and fig. 3.3). Further, no significant difference of the calculated maximum stress values and of the respective AT stress-strain relationship between the non-dominant and dominant leg was found (tab. 3.1 and fig. 3.2).

Tab. 3.1 Investigated parameters (mean ± standard deviation) of the non-dominant and dominant leg and the corresponding correlation coefficient (r) between sides (n = 36).

Parameter	Non-dominant	Dominant	r
Moment [Nm]	234 ± 44	234 ± 38	0.73⁺
Force [N]	4399 ± 694	4436 ± 726	0.66⁺
Elongation [mm]	14.2 ± 2.3	14.0 ± 2.5	0.54⁺
Lever arm [mm]	53.4 ± 6.7	53.2 ± 6.3	0.90⁺
Stiffness [N/mm]	320 ± 113	339 ± 114	0.45⁺
Rest length [mm]	202.2 ± 25.3	212.5 ± 27.7*	0.89⁺
Strain [%]	7.1 ± 1.4	6.7 ± 1.5	0.38⁺
Emodulus [GPa]	0.82 ± 0.25	0.93 ± 0.33*	0.46⁺
CSA [mm²]	78.7 ± 12.3	78.9 ± 12.1	0.78⁺
Length [mm]	52.2 ± 16.7	56.5 ± 17.1*	0.89⁺
Stress [MPa]	57.0 ± 11.7	57.0 ± 11.4	0.69⁺

Moment is referring to the maximum plantar flexion moment, force is the maximum calculated tendon force, elongation is the maximum elongation of the Achilles tendon (AT), lever arm is the AT lever arm, stiffness is AT stiffness, rest length is defined as the distance from the tuberositas calcanei to m. gastrocnemius medialis-Achilles tendon junction, strain is the maximum strain of the AT, Emodulus is the Young's modulus of AT, CSA is the average cross-sectional area, length is the length of the free AT (m. soleus-AT junction to initial attachment on the calcaneus bone) and stress is the maximum AT stress.
* : statistically significant difference between the non-dominant and dominant side ($p < 0.05$)
⁺ : statistically significant correlation ($p < 0.05$)

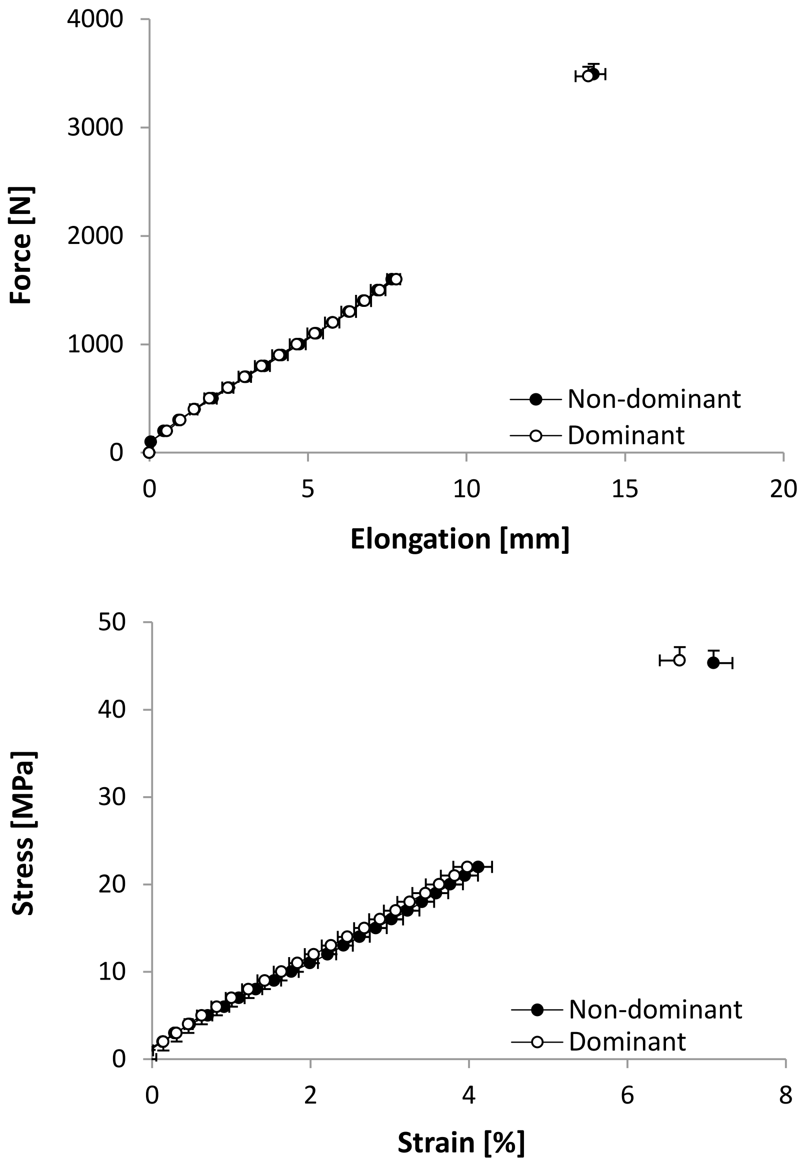

Fig. 3.2 Achilles tendon force-elongation relationship (every 100 N) and Achilles tendon stress-strain relationship (every 1 MPa) (mean ± standard error of mean) of the non-dominant and dominant leg during maximum voluntary contraction. The curves end at 1600 N and 22 MPa, which corresponds to the maximum common force and stress achieved by all participants and sides during the maximum voluntary contractions, respectively (n = 36).

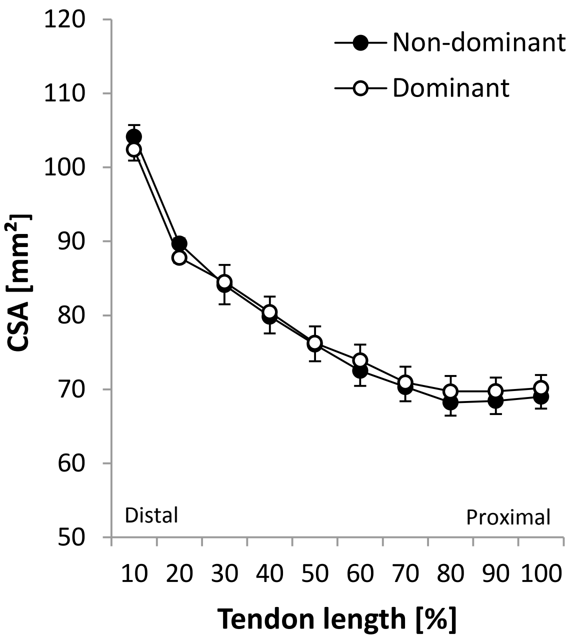

Fig. 3.3 Cross-sectional area (CSA) values (mean ± standard error of mean) of the free Achilles tendon from the non-dominant and dominant leg at every 10 % of tendon length (n = 36).

The correlation coefficients of the investigated parameters of the non-dominant and dominant leg ranged from r = 0.38 (maximum tendon strain) to r = 0.90 (AT lever arm; tab. 3.1), indicating a medium to high relationship of both sides. The AAI ranged from 3% to 31% (fig. 3.4) while the maximum moment (8.4 ± 8.2% (mean ± SD)), AT lever arm (2.9 ± 3.0%), AT rest length (6.4 ± 4.0%), and AT CSA (7.5 ± 5.9%) showed relative lower AAI values compared with maximum AT force (9.2 ± 7.8%), maximum AT elongation (12.7 ± 10.9%), AT stiffness (28.3 ± 17.2%), maximum AT strain (16.5 ± 11.3%), AT Young's modulus (31.1 ± 20%), length of free AT (12.7 ± 11.8%), and maximum AT stress (13.4 ± 9.5%) indicating low symmetry between legs for most of the investigated parameters. The non-dominant to dominant leg agreement of all investigated parameters of the m. triceps surae and AT is illustrated by the Bland and Altman plots in fig. 3.5. Except the limits of agreement of the lever arm, AT CSA, and rest length, for all parameters, the limits were higher than +20% and lower than −20% in relation to the respective mean value, presenting a poor agreement of these AT parameters of the non-dominant and dominant leg.

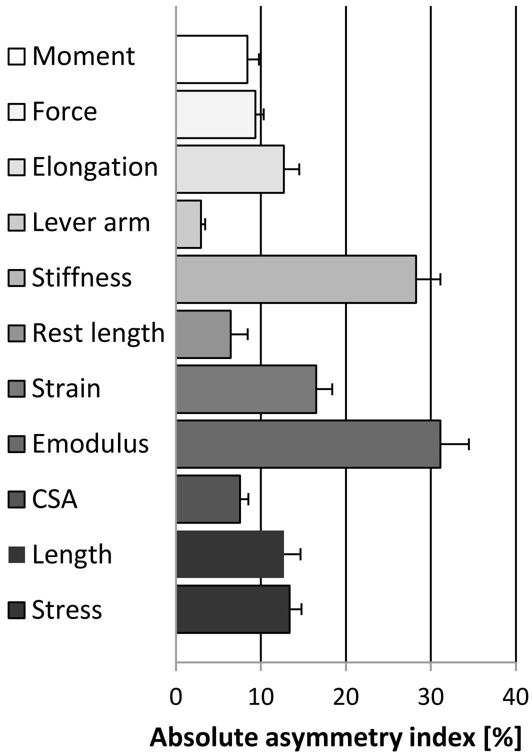

Fig. 3.4 Absolute asymmetry index for the investigated parameters of the Achilles tendon and triceps surae muscle of the non-dominant and dominant leg ($n = 36$). Moment is referring to the maximum plantar flexion moment, force is the maximum calculated tendon force, elongation is the maximum elongation of the Achilles tendon (AT), lever arm is the AT lever arm, stiffness is AT stiffness, rest length is defined as the distance from the tuberositas calcanei to m. gastrocnemius medialis-Achilles tendon junction, strain is the maximum strain of the AT, Emodulus is the Young's modulus of AT, CSA is the average AT cross-sectional area, length is the length of the free AT (m. soleus-AT junction to initial attachment on the calcaneus bone) and stress is the maximum AT stress.

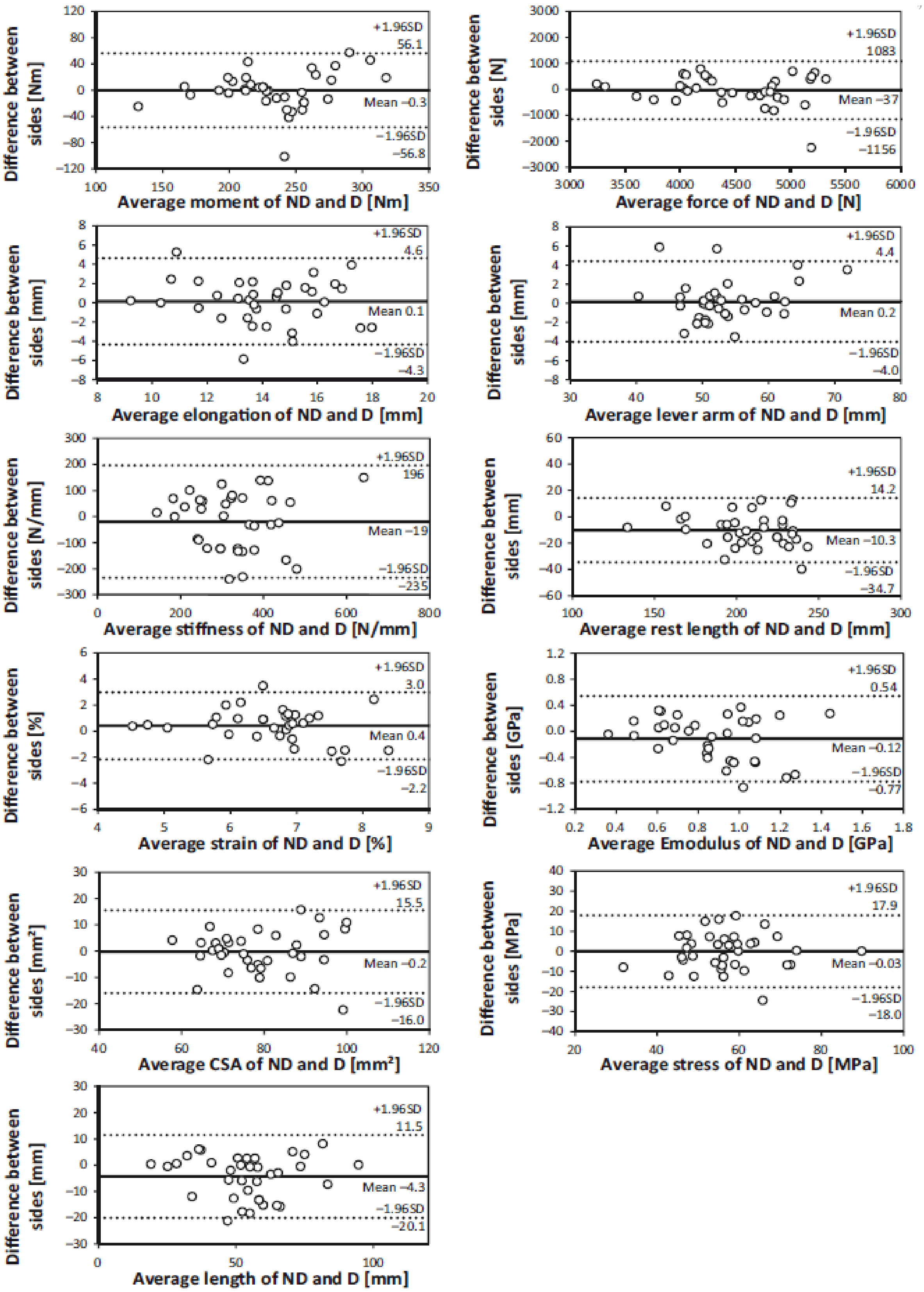

Fig. 3.5 Bland and Altman plots depicting the agreement of the non-dominant (ND) and dominant (D) leg for the moment (maximum plantar flexion moment), force (maximum tendon force), elongation (maximum elongation of the Achilles tendon (AT)), lever arm (AT lever arm), stiffness (AT stiffness), rest length (distance from the tuberositas calcanei to m. gastrocnemius medialis-AT junction), strain (AT maximum strain), Emodulus (AT Young's modulus), CSA (average AT cross-sectional area), length (length of the free AT (m. soleus-AT junction to initial attachment on the calcaneus bone)) and stress (maximum AT stress) (n = 36).

3.5. Discussion

The present study investigated the AT properties of the non-dominant and dominant leg with respect to symmetry. The findings showed a significantly higher (~ 14%) AT Young's modulus of the dominant leg compared with the non-dominant accompanied by a tendency toward lower maximum AT strain (~ 6%) of the dominant leg during the maximum contractions. The free AT length and rest length were significantly longer by 8% and 5% on the dominant compared with the contralateral side, respectively. Furthermore, the asymmetry index ranged from 3% to 31% within the examined parameters, indicating only moderate symmetry between sides. Moreover, Bland and Altman plots illustrated low agreement between the mechanical AT properties of the non-dominant and dominant leg, with limits of agreement for stiffness from +59 to −71% and for Young's modulus from +62 to −88% of the mean value and moderate agreement between the AT morphological properties of the non-dominant and dominant leg, with limits for average CSA from 20 to −20% and for the AT lever arm from 8 to −8% of the mean value. Therefore, we confirmed our hypothesis of asymmetrical AT properties between non-dominant and dominant leg in normally active individuals.
Tendons can adapt in response to mechanical loading that is applied during daily activities by altering their material (i.e., Young's modulus) (Reeves et al., 2003; Arampatzis et al., 2010) and/or morphological properties (i.e., CSA) (Arampatzis et al., 2007a; Kongsgaard et al., 2007; Seynnes et al., 2009). Such adaptation of tendons depends on the intensity of the performed activity (Kongsgaard et al., 2005; Arampatzis et al., 2007b). Specific activities may induce higher mechanical loads to either the AT of the non-dominant and dominant leg, which might in turn affect the tendon properties. Therefore, dissimilar mechanical loading profiles of the non-dominant and dominant leg during daily life as previously reported (Valderrabano et al., 2007; Wang & Watanabe, 2012) seems to be responsible for the observed differences of the tendon properties between both legs. The finding of a higher AT Young's modulus of the dominant compared with the non-dominant leg in our study may be explained by asymmetric loading as a result of foot preference. In agreement with this rationale, the AT lever arm, as an anthropometric parameter that is not influenced by daily loading, did not significantly differ between sides and the AAI was comparatively low (3%). The CSA did not differ significantly between the AT of the non-dominant and dominant leg and AAI was notably lower compared with Young's modulus (8% and 31%, respectively). It seems that different daily loading profiles of the non-dominant and dominant leg affected primarily the tendon material properties rather than morphological properties (i.e., CSA). Several studies found an increase in tendon Young's modulus without changes in the tendon CSA following training interventions (Kubo et al., 2001;

Reeves et al., 2003; Arampatzis et al., 2010), indicating that material properties may be more sensitive and instant in responding to increased mechanical loads. These reports are in good agreement with our suggestion that side-dependent adaptations to different daily loading profiles (i.e., foot dominance) may have been responsible for the asymmetry we found primarily on the material properties.

The maximum plantar flexion moment, AT force, AT elongation, AT stiffness, AT strain, AT lever arm, AT CSA, and AT stress did not significantly differ between the non-dominant and dominant leg. However, the correlation coefficients for theses parameters ranged from r = 0.38 to r = 0.78 (except the AT lever arm with r = 0.90), indicating a moderate association between the two legs. Regarding the mentioned parameters, the AAI ranged from 8% to 28% (except the lever arm with 3%), indicating that the values of non-dominant and dominant leg were not symmetrically although statistical significant differences of theses parameters between sides were not detected. Furthermore, the Bland and Altman plots illustrate these asymmetrical distributions of the parameter of the non-dominant to the dominant leg by the "limits of agreement" interval that is based on the standard deviation of the differences between the non-dominant and dominant leg and contains 95% (two times standard deviation) of these differences. In relation to the respective mean average value, the limits of agreement of the investigated parameters ranged from +20% to −20% and +59% to −71%, indicating a poor side agreement.

For the finding of significant longer free AT length (8%) and rest length (5%) on the dominant compared with the contralateral non-dominant leg, we cannot give a clear explanation by means of the applied experimental design regarding the possible responsible mechanisms. However, in accordance to our results, Pang and Ying (2006) reported comparable differences of the free AT length of the non-dominant compared with the dominant leg. Furthermore, Balius et al. (2013) reported similar differences between the left and right free AT length in a cadaver study on 11 human specimens. In agreement with the present results, the left AT was the shorter one (33 of 36 participants defined their left leg as non-dominant).

The AT properties were not symmetrical between the non-dominant and dominant leg and, thus, a general transferability from one to the other leg seems to be doubtful. However, when AT properties are impaired because of pathologies such as rupture or tendinopathy (Arya & Kulig, 2010; Helland et al., 2013); the healthy contralateral leg is often used to serve as a reference to quantify the pathological alterations (Silbernagel et al., 2006; Couppé et al., 2013; McNair et al., 2013). This approach is based on the assumption that the tendon properties of the healthy and pathological side are more or less similar in a healthy state. In contrast, the findings of our study demonstrated differences between the properties of the AT of both legs in a healthy population. This indicates that a comparison between a pathological and a healthy side should be taken with care, as differences in the AT properties may falsely be interpreted as a result of pathological deficits rather than sidedness effects. Therefore, the utilization of the healthy contralateral leg as a reference for the identification of pathological or regenerative alterations in tendon properties might be questionable a priori. Moreover, from a methodological point of view, cross-sectional studies investigating tendon properties on only one leg on different samples (e.g., active vs. inactive subjects) should consider that tendon properties could differ between legs to avoid potential effects of laterality on the sample comparison.

In conclusion, the present study showed clear asymmetries of AT properties between the non-dominant and dominant leg in a normally active healthy population. The observed asymmetry of the tendon properties of the non-dominant and dominant leg may be a result of different loading profiles (foot dominance) of both legs during daily activities.

3.6. Perspective

The findings our study showed an asymmetry of AT properties between the non-dominant and dominant leg in a normal active healthy population. Therefore, a general transferability from the AT properties of one to the contralateral leg seems illegitimate. In a clinical setting of tendon pathologies, a comparison of the healthy and affected leg is often used to quantify the pathological or regenerative state of the affected leg (Silbernagel et al., 2006; Couppé et al., 2013; McNair et al., 2013). However, as shown by our results, this comparison is not appropriate because symmetry of AT properties between sides in our healthy population was not evident. Therefore, we recommend referring to matched control groups rather than the healthy contralateral leg. However, to investigate adaptive responses of the tendon following an intervention a pre-post comparison of the same leg could be more accurate. Furthermore, future cross-sectional studies should consider potential differences of tendon properties between non-dominant and dominant leg and therefore, between left and right leg in their study design. Instead of measuring only one leg, it might be recommended to either measure both legs or check for foot preference in advance to avoid possible effects of asymmetry on the sample comparison.

3.7. Acknowledgements

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4. Second study:

Chronic Mechanical Loading and Tendon Adaptive Responses: A Systematic Review and Meta-Analysis

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4.1 Abstract

Background:

Tendons transmit the force exerted by a muscle to the bone and, therefore, are crucial for human locomotion. Increasing the mechanical load, tendons respond with a change of their properties, which allow for improvements of athletic performances as well as injury prevention and therapy.

Objective:

The present article systematically reviews recent literature on the in vivo adaptation of human tendon mechanical (i.e. stiffness), material (i.e. Young's modulus) and morphological (i.e. cross-sectional area) properties to chronic loading, and meta-analyses the loading conditions, intervention outcomes as well as methodological aspects.

Data sources:

The search was performed in the databases Pub Med, ISI Web of Knowledge and Scopus (1970 to April 2014) and by screening the reference lists of the eligible articles.

Study eligibility criteria:

A study was included if reporting (a) a longitudinal (≥8 weeks) exercise intervention on (b) healthy humans (18-50 yr), (c) investigating the effects on mechanical (stiffness), material (Young's modulus) and/or morphological (cross-sectional area) properties of tendons in vivo.

Study appraisal and synthesis methods:

Weighted average effect sizes of the intervention-induced adaptations of the tendon stiffness, Young's modulus and cross-sectional area were calculated using a random-effects model and tested for an overall intervention effect. Heterogeneity between studies was checked using Q and I2-statistics. The methodological quality of each study was assessed by means of a customized scale and the risk of bias according to the Cochrane risk of bias tool.

Results:

The review process yielded 26 studies with 33 separate exercise interventions on either the Achilles or patellar tendon including in total 317 participants. The weighted average effect size was 0.66 (confidence interval: 0.46, 0.87) for tendon stiffness (n=33 studies), 0.66 (0.23, 1.10) for Young's modulus (n=13) and 0.24 (0.04, 0.45) for cross-sectional area (n=26), featuring significant overall intervention effects ($p<0.05$). The heterogeneity of the assessed interventions

was significant ($p<0.05$) for tendon stiffness ($I^2=33\%$) and Young’s modulus ($I^2=64\%$) and in tendency for cross-sectional area ($p=0.06$, $I^2=31\%$), indicating that differences in the loading conditions may affect the adaptive responses. The methodological quality assessment showed an average score of 70 ± 8% (range 61 to 92%), which represents an appropriate methodological quality for most studies. The risk of bias assessment was heavily restricted by a general lack of relevant information.

Limitations

The sample sizes of all included studies were relatively small ($n<16$), the participants mostly male (291 of 317), involved in recreational activity (approximately 255 of 317) and the intervention durations short-term (≤14 weeks, except two studies).

Conclusions

The present meta-analysis provides elaborate statistical evidence that tendons are highly responsive to diverse loading regimens. However, data strongly suggests that loading magnitude in particular may play a key role for tendon adaptation. Furthermore, intervention-induced changes in tendon stiffness seem to be more attributed to adaptations of the material rather than morphological properties.

Key words:

tendon training, tendinous tissue plasticity, tendon function adaptation

4.2 Introduction

Tendons transmit the force exerted by the corresponding muscle to the skeleton and, therefore, are crucial components for human locomotion [1–3]. Further, the non-rigidity of tendons allows the storage and return of strain energy during locomotion [4,5] and facilitates the muscle force potential due to the force-length-velocity relationship [6–8]. Hence, tendon properties effect human daily locomotion like walking/running [9] and stability performance [10], but also significantly determine athletic performances, e.g. sprinting [11,12] and jumping [8,13,14] as well as the economy of running [15–17]. Furthermore, tendons are sensitive to their mechanical environment [18–22]. Following a period of enhanced mechanical loading, tendon stiffness may increase [23–26] to maintain physiological ranges of strain during locomotion, since the ultimate tendon strain is more or less constant [27]. Two mechanisms could account for an increase of tendon stiffness: a) changes of the tendon material (i.e. increase of Young's modulus) and b) changes of the tendon morphological properties (i.e. increase of cross-sectional area) [24,28–30]. Both, tendon material and morphological changes result from an increase of collagen synthesis, but also from changes of collagen fibril morphology and levels of collagen molecular cross-linking [19,31,32].

The development and improvement of measurement techniques in the past 15 years, especially the measurement of tendon elongation during muscle contractions by means of an ultrasound-based methodology as well as the determination of the tendon cross-sectional area (CSA) from magnetic resonance images (MRI), enabled researchers to investigate human tendon mechanical, material and morphological properties in vivo and adaptive responses following chronic loading [2,19,33]. Kubo et al. [34] were the first who reported an increase in stiffness and Young's modulus of the patellar tendon in humans following twelve weeks of exercise-based loading. An intervention-induced region specific hypertrophy of the patellar and Achilles tendon were initially reported in 2007 by Kongsgaard et al. [24] and Arampatzis et al. [29], respectively. To date, a lot of experimental studies evidenced the adaptive potential of tendons following exercise interventions, which featured different levels of mechanical loading conditions (e.g. intensity, duration, repetitions, sets, intervention duration and training frequency per week) [25,28,30,35–38]. Since some interventions reported greater adaptive tendon responses than others, the outcome of the studies seems to be affected by differences of the applied loading conditions. This means that the levels of the loading conditions may determine the material and morphological adaptive responses of tendons. Although some studies investigated the effect of different loading levels (i.e. load magnitude [24,29] and load duration [34,39]) on tendon adaptation, the small sample sizes of 8-12 participants used in these studies limits the generalizability of the outcomes. A meta-analysis of relevant experimental studies that examines the interaction of the levels of loading conditions with respect to study outcome could deepen our understanding of the effectiveness of certain loading levels on tendon adaptation. Furthermore, different methodological approaches could have affected the study outcomes, thus, additionally challenging the generalization of the findings. For example, using the ultrasound technique instead of MRI to measure the tendon CSA [35,36,40,41], intervention-induced changes of the tendon CSA might have been undetected or overrated, since the reliability of this method was reported to be poor [42]. Considering the methodological quality (i.e. internal, statistical, external validity aspects) of each study in a systematic meta-analysis would further improve our knowledge regarding mechanical loading and tendon adaptation.

Therefore, the objective of the present study is to systematically review recent literature reports on human tendon adaptation following chronic mechanical loading in vivo and to meta-analyse the applied levels of loading conditions, intervention outcomes as well as methodological aspects, which has not been conducted thus far. This meta-analysis may provide crucial information on how to facilitate tendon adaptation. For a complete description of the adaptive processes, we will consider tendon mechanical, material and morphological properties.

4.3 Methods

4.3.1 Search strategy

The search was performed by using the electronic bibliographic databases ISI Web of Knowledge, Pub Med and Scopus (1970 to April 2014) and by screening the reference lists of the eligible articles. The following keyword combinations were applied in the database search: tendon properties adaptation, tendon stiffness adaptation, tendon function adaptation, tendon mechanical loading adaptation, tendon properties training and tendon properties exercise.

4.3.2 Study selection and inclusion criteria

Two independent reviewers evaluated the titles of the studies that resulted from the search and included studies when the title indicated that the following inclusion criteria were fulfilled: (a) a longitudinal (≥8 weeks) exercise intervention was conducted, (b) healthy humans (18-50 yr) served as participants and (c) the effects on mechanical (stiffness), material (Young's modulus) and/or morphological (CSA) properties of tendons in vivo were reported (d) in English language. The abstracts and, thereafter, the full text of the identified studies were then examined to confirm the inclusion. If a study did not meet all criteria, the respective exclusion criterion was documented and the study was eliminated from further analysis. In the case of disagreement of the two reviewers, a third reviewer was consulted. Figure 4.1 illustrates the systematic review process of the present meta-analysis.

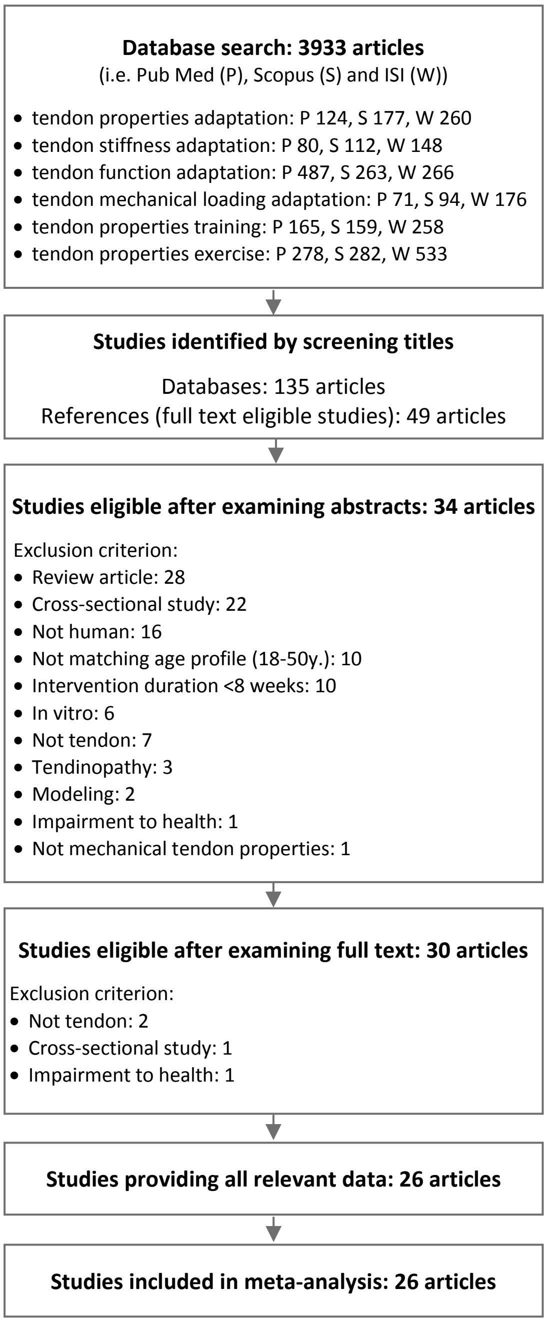

Fig. 4.1 Flowchart of the systematic review process

4.3.3 Methodological quality and risk of bias

A customized methodological quality scale was designed to assess the internal, statistical and external validity of the included studies in regard to the conceptual definition (tab. 4.1). A positive point was assigned when a specific quality criterion was fulfilled (tab. 4.1). However, if a criterion could not be scored because it was not part of the study (e.g. mechanical but not morphological tendon properties were investigated), the criterion was excluded from the further quality assessment of the study. The quality score of each validity aspect (i.e. internal, statistical and external) was calculated by dividing the number of items with a positive score by the total number of items (the quotient was then multiplied by 100). The scores were averaged to calculate the overall methodological quality of each study. The assessment of the risk of bias (sequence generation, allocation concealment, blinding outcome assessor, incomplete outcome data, selective outcome reporting, other sources of bias) was based on the Cochrane Risk of Bias tool [43]. The data extraction and scoring were performed by two independent observers and, in the case of disagreement, a third one was consulted.

4.3.4 Data extraction

One reviewer extracted the relevant data from the full text articles of all included studies and a second reviewer confirmed the extraction. The data were merged in a table, including the following information: the name of the first author and year of publication, the label of the participant sample in the respective study (i.e. experimental or control group according to the inclusion criteria), the number, gender and activity level of the participants, the investigated tendon, the type of the performed training with the respective loading conditions and the outcome of the study for either tendon stiffness, Young's modulus and CSA as the difference of pre and post values in percentage (i.e. ((post value - pre value) / pre value) * 100) with the corresponding significance level (i.e. p-values). Furthermore, the part of the tendon (i.e. free tendon or tendon-aponeurosis-complex) that was used for the parameter calculation was documented. In studies where both calculation approaches were used, only the values from the free tendon were included. If the stiffness or Young's modulus was calculated within several percentage intervals of the force-elongation or stress-strain-relationship, the values from the highest interval were used. In case that the CSA was reported for different positions along the tendon length, the mean value and pooled standard deviation was calculated and included. If the required data (i.e. means and standard deviations of pre and post intervention values) were not reported in the article or presented in an inappropriate format for data extraction (e.g. graph instead of values), the corresponding authors were contacted and asked to provide the missing values. Extracting values visually from a graph was the final option. In case the relevant data were not available, the study was excluded.

Tab. 4.1 Criteria of the methodological quality

Internal validity	Scoring
1. Study design	A positive point was assigned if the following aspects were considered: 1 Mechanical tendon properties (stiffness) 2 Material tendon properties (Young’s modulus) 3 Morphological tendon properties (cross-sectional area) 4 Control group (no specific training) was included and participants were randomly assigned
2. Methods	A positive point was assigned if the following aspects were considered:
2.1 Mechanical properties • Object of investigation	A Only the free tendon was assessed [62]
• Calculation of tendon force	B Consideration of gravitational forces [63,64] C Consideration of axes misalignment of dynamometer and joint [63–65] D Consideration of antagonistic muscle activation [66,67] E Tendon lever arm directly measured for each subject
• Measurement of tendon elongation	F Consideration of joint angle changes during the maximal isometric contraction on the tendon elongation measurement [67,68] G Using the average of multiple trials (>1) to increase the reliability of the ultrasound technique [69]
2.2 Morphological properties	A Magnetic resonance imaging was used [42,70] B Different positions along tendon length were assessed to account for potential region specific adaptations [24,29,30]
3. Cofactors	A positive point was assigned if the following aspects were considered: A Influence of gender B Influence of physical activity level of the participants
Statistical validity	Scoring
4. Statistical tests	A positive point was assigned if appropriate statistical tests were used
5. Power analysis	A positive point was assigned if effect sizes were calculated and reported
External validity	Scoring
6. Eligibility of sample and variable	A positive point was assigned if the intervention included: 1 Appropriate participant sample 2 Appropriate variables
7. Description of the exercise intervention protocol	A positive point was assigned if the following criteria were reported: A Intensity of muscle contraction B Duration of single stimulus C Repetitions per set D Number of sets E Number of weeks of intervention F Number of trainings per week
8. Description of the participant sample	A positive point was assigned if the following criteria were reported: A Age, B Gender, C Body height, D Body weight, E Activity level

4.3.5 Statistical analysis

In order to assess the impact of mechanical loading on tendon adaptation, the effect sizes of the intervention-induced changes (i.e. changes to baseline) of the tendon stiffness, Young's modulus and CSA for each study were calculated. As the stiffness, Young's modulus and CSA were not always measured using identical methodological approaches, the effect size was calculated as the standardized mean difference (SMD) [44]. The SMD included further an adjustment (Hedges' adjusted g) for small sample bias [44]. The effects sizes from all studies were then pooled in a meta-analysis to estimate the weighted average effect size of the tendon stiffness, Young's modulus and CSA [44,45]. Thereto, we used a random-effects model of the generic inverse variance method, which gives more weight to larger studies (i.e. smaller standard errors) and account for heterogeneity of the included studies [44,46]. To check for the presence of an overall intervention effect on the tendon stiffness, Young's modulus and CSA a test statistic (i.e. null hypothesis: no overall effect of the intervention) was performed [44]. A forest plot was created to illustrate the effect sizes and 95% confidence intervals (CI) of tendon stiffness, Young's modulus and CSA for all respective studies as well as the overall effect. Further, heterogeneity between studies was tested using Q and I2-statistics to assess the variation due to study heterogeneity rather than chance [47]. Statistical procedures were performed by means of the software Review Manager v.5.2 [48].

4.4 Results

4.4.1 Literature search

The search by the defined keywords yielded 3933 hits in the three databases (fig. 4.1). After screening all study titles and eliminating duplicates from the different databases, 135 potentially eligible studies were identified. Following the abstract examination 33 studies remained included, however, the full text assessment showed that four more studies did not confirm all criteria and, thus, were excluded from the further analysis. The screening of the reference lists of the included studies provided a number of 49 potentially eligible studies. However, except one study all articles did not meet the criteria or were already included. Four studies were excluded from the remaining 30 due to a lack of relevant information about the loading conditions [49] or outcome values [41,50,51]. Finally, 26 studies fulfilled all criteria and were included in the present meta-analysis (fig. 4.1).

4.4.2 Description of included studies

All included studies assessed the effect of mechanical loading on either the patellar tendon (n=12) or the Achilles tendon (n=14). Eight studies applied a different loading protocol on the two legs of the participants of the exercise group and one study investigated three different intervention groups. In the present meta-analysis, each of these interventions was treated as a separate intervention. When a study presented the data of different intervention groups but not all of them fulfilled the inclusion criteria, only the ones which met all criteria were included. The articles from Foure et al. [35,52,53] reported the effect of a single intervention on different parameters of the Achilles tendon. The relevant parameters for the present analysis (i.e. tendon stiffness and CSA) were extracted and considered as a single intervention. Furthermore, Kubo et al. [34,39] presented the data of one intervention in two articles, as indicated by the same number of participants and anthropometrics, training protocol and results of tendon stiffness and CSA (LC protocol exercise group in [39]). Thus, the values were only included once. In the study of Kubo et al. [54], the authors compared the results of two previous investigations [55,56] that were already included in the present meta-analysis under a new research question. These results were also not considered as a new investigation. In another article [24], the CSA pre and post intervention values were exclusively reported in a graph (figure 4, page 116). The respective means and standard error of means were visually extracted from the graph and used in order to calculate the standard deviation and effect size.

Finally, the present meta-analysis included in total 33 interventions (participants in total n=317) eligible for the research question and their characteristics were summarized in table 4.2. In all 33 interventions the parameter tendon stiffness was used in order to quantify the training effect on the adaptive tendon responses. Twenty-nine of these also examined the tendon CSA and 13 studies further included the parameter Young's modulus. Fourteen interventions applied the mechanical stimulus on the tendon by means of isometric muscle contractions, ten interventions used a combination of concentric and eccentric contractions or solely concentric (n=1) or eccentric contractions (n=3), four performed plyometric training, one intervention added stretching to the resistance training and one study investigated the effect of running on the tendon properties (tab. 4.2). The loading conditions were set to different levels between studies, using high and low intensities, short and long durations of the single load and different numbers of repetitions and sets (tab. 4.2). However, only two studies (i.e. four interventions) specified the corresponding tendon strain magnitude to the muscle contraction intensity [28,29]. Thirty-one of the 33 interventions were performed for 8 to 14 weeks and the participants exercised on two to four days per week. Except four interventions [29,57,58], which included both, female and male participants and one intervention including solely women [59], all other interventions were performed with men. In almost all studies the participants were regularly physically active, but not involved in intensive sports activity. One intervention was performed with Cricket players [36] and another one with runners [17]. The number of exercised participants ranged between studies from six to 15 with a mean of 9.6 ± 2.0.

4.4.3 Methodological quality assessment

The results of the methodological quality assessment of the included studies showed a range of achieved scores from 61 to 92% with a mean and standard deviation of 70 ± 8% (tab. 4.3), indicating appropriate methodological qualities for most studies. Thirteen of the 26 included studies investigated mechanical, material as well as morphological properties (i.e. stiffness, Young's modulus and CSA), which is essential in order to clarify if a change in tendon stiffness was based on alterations of the material properties and/or tendon hypertrophy.
The risk of bias assessment indicated a low risk of bias in three interventions [24,38,57]. The judgment for the other included studies was problematic, because the randomization process, concealment of allocation and blinding of the assessor to the data were not reported and, therefore, unclear (tab. 4.3).

4.4.4 Meta-analysis of intervention effects

The weighted average effect size for the tendon stiffness was 0.66 (CI 0.46, 0.87), 0.66 (CI 0.23, 1.10) for tendon Young's modulus and 0.24 (CI 0.04, 0.45) for tendon CSA, indicating greater intervention effects on stiffness and Young's modulus compared to CSA (fig. 4.2). The overall intervention effect was significant for all three parameters ($p<0.05$).
Heterogeneity was significant for stiffness and Young's modulus ($p<0.05$) and in tendency for CSA ($p=0.06$), with a moderate heterogeneity of 33% and 31% for stiffness and CSA, respectively, and a substantial heterogeneity of 64% for Young's modulus [47].
Figure 4.2 presents a forest plot, including the effects sizes and corresponding confidence intervals for tendon stiffness, Young's modulus and CSA of all included interventions as well as the respective weighted average effect sizes with the overall effect test and heterogeneity analysis results.

Tab. 4.2 Data extraction from the included studies

Study			Participants			Intervention								Outcome							
Reference	Year	Group	N	Sex	Activity level	Tendon	Type of training	Intensity	Duration	Reps	Sets	Weeks	x/ week	Stiffness Location	Stiffness %	Stiffness Sig	YM %	YM Sig	CSA Location	CSA %	CSA Sig
Albracht et al. [15]	2013	EP	13	m	Run	AT	Is (rep)	90% MVC	3s	4	5	14	4	Ap (GM F)	15.8	*					
Arampatzis et al. [29]	2010	EP	11	m	Reg	AT	Is (rep)	55% MVC	1s	20	5	14	4	Ap (GM F)	-5.2	-	-4.8	-	Free	1.3	-
		EP	11	m	Reg	AT	Is (rep)	90% MVC	1s	12	5	14	4	Ap (GM F)	17.1	*	16.9	*	Free	0.5	-
Arampatzis et al. [28]	2007	EP	11	f,m	Reg	AT	Is (rep)	55% MVC	3s	7	5	14	4	Ap (GM F)	7.9	-	-1.6	-	Free	4.3	-
		EP	11	f,m	Reg	AT	Is (rep)	90% MVC	3s	4	5	14	4	Ap (GM F)	36.0	*	22.9	*	Free	9.6	*
Carroll et al. [57]	2011	CG	7 (11)	f,m	Unt	PT	Co-Ec (rep)	74% RM	nr	2-3	5-10	12	3	Free	13.9	+	18.4	*	Free	-1.7	-
Fletcher et al. [17]	2010	EP	6	m	Run	AT	Is (sta)	80% MVC	20s	1	4	8	3	Ap (GM F)	18.6	-					
Fouré et al. [61]	2009	EP	6	m	Exp	AT	Ply	nr	nr	150-280	nr	8	2	Ap (GM M)	4.1	-					
Foure et al.[35,52,53]	2010a,b,2011	EP	9	m	Reg	AT	Ply	nr	nr	200-600	nr	14	2.4	Ap (GM M)	26.5	*			Free	3.1	-
Foure et al. [40]	2013	EP	11	m	Reg	AT	Ec (rep)	nr	nr	200-600	nr	14	2.4	Ap (GM F)	16.4	-			Free	-1.5	-
Hansen et al. [58]	2003	EP	11	f,m	Unt	AT	Run	nr	30-50 min	1		34	2.4	Ap (GM F)	7.3	-			Free	-0.3	-
Houghton et al. [36]	2013	EP	7	nr	Cri	AT	Ply	nr	nr	4-10	2-6	8	1.9	Ap (GM M)	-8.9	-	-20	-	Free	12.9	*
Kongsgaard et al. [24]	2007	EP	12	m	Unt	PT	Co-Ec (rep)	70% RM	nr	8	10	12	3	Free	14.6	*	12.2	-	Free	3.3	nr
		EP	12	m	Unt	PT	Co-Ec (rep)	16% RM	nr	36	10	12	3	Free	-9.2	-	-4.2	-	Free	1.5	nr
Kubo et al. [34,39]	2001a	EP	8	m	Reg	PT	Is (rep)	70% MVC	rapid	50	3	12	4	Ap (VL F)	17.5	-			Free	1.4	-
	2001a, b	EP	8	m	Reg	PT	Is (static)	70% MVC	20s	1	4	12	4	Ap (VL F)	57.3	*	50.3	*	Free	1.4	-
Kubo et al. [25]	2002	EP	8	m	Reg	AT	Co-Ec (rep)	70% RM	nr	10	5	8	4	Ap (GM F)	31.3	*			Free	-3.3	-
		EP	8	m	Reg	AT	Co-Ec (rep) + S	70% RM	nr + 45s	10 + 5	5 +1	8	4+7 (2x/d)	Ap (GM F)	23.8	*			Free	3.4	-
Kubo et al. [59]	2003	EP2	11	f	Reg	PT	Co-Ec (rep)	BW	nr	44	1	24	6	Ap (VL F)	15.7	-					
Kubo et al. [37]	2006a	EP	9	m	nr	PT	Is (sta) [50°]	70% MVC	15s	1	6	12	4	Ap (VL F)	9.7	-			Free	1.5	-
		EP	9	m	nr	PT	Is (sta) [100°]	70% MVC	15s	1	6	12	4	Ap (VL F)	50.9	*			Free	1.5	-
Kubo et al. [71]	2006b	EP	8	m	Reg	PT	Is (sta)	70% MVC	15s	1	10	12	4	Free	-0.2	-			Free	0.3	-
Kubo et al. [72]	2006c	CG	9	m	nr	PT	Co-Ec (rep)	80% RM	4s	10	4	12	3	Free	8.5	-			Free	-0.6	-
Kubo et al. [55]	2007	EP	10	m	Unt	AT	Ply	40% RM	nr	10	5	12	4	Ap (GM M)	19.4	-			Free	3.3	-
		EP	10	m	Unt	AT	Co-Ec (rep)	80% RM	4s	10	5	12	4	Ap (GM M)	29.7	*			Free	-1.2	-
Kubo et al. [56]	2009	EP	10	m	nr	PT	Is (static)	70% MVC	15s	1	10	12	4	Free	71.1	*			Free	4.0	-
		EP	10	m	nr	PT	Co-Ec (rep)	80% RM	4s	10	5	12	4	Free	25.4	-			Free	1.3	-
Kubo et al. [49]	2010	EP	8	m	Reg	PT	Is (sta)	70% MVC	15s	1	10	12	4	Ap (VL F)	50.9	*			Free	1.0	-
Kubo et al. [73]	2012	EP	9	m	Reg	AT	Is (sta)	80% MVC	15s	1	15	12	4	Ap (GM M)	51.4	*			Free	2.7	-
Malliaras et al. [38]	2013	EP	9	m	Reg	PT	Co (rep)	80% RM	5s	7-8	4	12	3	Free	49.9	-	52	-	Free	5.0	-
		EP	10	m	Reg	PT	Ec (rep)	80% RM	5s	12-15	4	12	3	Free	39.2	-	38.6	-	Free	3.6	-
		EP	10	m	Reg	PT	Ec (rep)	80% RM(ex)	5s	7-8	4	12	3	Free	80.9	*	77.3	*	Free	5.8	-
Seynnes et al. [30]	2009	EP	15	m	Reg	PT	Co-Ec (rep)	80% RM	nr	10	4	9	3	Free	22.7	*	18.4	*	Free	3.9	*

Abbreviations: **Group** (i.e. as assigned in the respective article) **|** EP: Experimental group | CG: Control group | **Sex |** f: Female | m: Male | **Activity level |** Reg: Regularly physical active and recreational sports |Unt: Untrained | Exp: Explosive sports (i.e. volleyball, basketball, handball) | Run: Runners | Cri: Cricket players | **Tendon |** PT: Patellar tendon | AT: Achilles tendon | **Type of training |** Is: Isometric muscle contraction | Co: Concentric | Ec: Eccentric | Ply: Plyometric | Run: Running | S: Stretching | rep: Repetitive | sta: Static | **Intensity |** MVC: Maximum voluntary contraction | RM: One repetition maximum | (ex): RM measured in eccentric condition | BW: Body weight | **Outcome |** YM: Tendon Young's modulus | CSA: Tendon cross-sectional area | **Location** (i.e. refers to the anatomical structure that was used for the assessment of the tendon properties)**|** Ap: Aponeurosis | GM: m. gastrocnemius medialis | VL: m. vastus lateralis | F: Fiber | M: Myo-tendinous junction | Free: Free tendon | nr: Not reported | **Sig** (i.e. significance) **|** * p<0.05 | + p<0.01 | - p>0.05

Tab. 4.3 Methodological quality and risk of bias assessment of the included studies

Study	Methodological quality																																	Risk of bias					
	Internal validity																Statistical validity			External validity													Total score	Sequence	Allocation	Blinding	Outcome	Report	Other
	1.1	1.2	1.3	1.4	2.1A	2.1B	2.1C	2.1D	2.1E	2.1F	2.1G	2.2A	2.2B	3A	3B	Score [%]	4	5	Score [%]	6.1	6.2	7A	7B	7C	7D	7E	7F	8A	8B	8C	8D	8E	Score [%]	[%]					
Albracht et al., 2013 [15]	+	-	-	+	-	+	+	+	-	+	-	/	/	-	+	51	+	-	50	+	+	+	+	+	+	+	+	-	+	+	+	+	95	65	Unclear	Unclear	Unclear	Yes	Yes	Yes
Arampatzis et al., 2007 [29]	+	+	+	+	-	+	+	+	-	+	-	+	+	+	-	87	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	-	95	77	Unclear	Unclear	Unclear	Yes	Yes	Yes
Arampatzis et al., 2010 [28]	+	+	+	-	-	+	+	+	-	+	-	+	+	+	-	72	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	-	95	72	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Carroll et al., 2011 [57]	+	+	+	-	+	-	-	-	-	+	+	+	+	-	+	70	+	-	50	+	+	+	-	+	+	+	+	+	+	+	+	+	96	72	Unclear'	Unclear'	Yes	Yes	Yes	Yes
Fletcher et al., 2010 [17]	+	-	-	+	-	+	+	-	+	+	-	/	/	+	+	60	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	70	Unclear	Unclear	Unclear	Yes	Yes	Yes
Fouré et al., 2009 [61]	+	-	-	+	-	+	-	-	-	-	-	/	/	+	+	46	+	-	50	+	+	-	-	+	-	+	+	+	+	+	+	+	88	61	Unclear	Unclear	Unclear	Unclear	Yes	Yes
Foure et al., 2010a, b, 2011 [35,52,53]	+	-	+	+	-	+	-	-	-	+	-	-	+	+	+	63	+	-	50	+	+	-	-	+	-	+	+	+	+	+	+	+	88	67	Unclear	Unclear	Unclear	Yes	Yes	Yes
Foure et al., 2013 [40]	+	-	+	+	-	+	-	-	-	+	-	-	+	+	+	63	+	-	50	+	+	-	-	+	-	+	+	+	+	+	+	+	88	67	Unclear	Unclear	Unclear	Yes	Yes	Yes
Hansen et al., 2003 [58]	+	-	+	-	-	-	-	+	+	+	-	+	+	-	+	56	+	-	50	+	+	-	+	+	+	+	+	+	+	+	+	+	96	67	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Hougthon et al., 2013 [36]	+	+	+	+	-	+	+	+	+	+	-	-	+	+	+	84	+	+	100	+	+	-	-	+	+	+	+	+	+	+	+	+	92	92	Unclear	Unclear	Unclear	Yes	Yes	Unclear⁺
Kongsgaard et al., 2007 [24]	+	+	+	-	+	+	-	+	-	+	-	+	+	+	+	74	+	-	50	+	+	+	-	+	+	+	+	+	+	+	+	+	96	73	Unclear'	Unclear'	Yes	Yes	Yes	Yes
Kubo et al., 2001a, b [34,39]	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	66	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	72	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Kubo et al., 2002 [25]	+	-	+	-	-	-	-	-	-	-	+	+	-	+	+	52	+	-	50	+	+	+	-	+	+	+	+	+	+	+	+	+	96	66	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Kubo et al., 2003 [59]	+	+	+	+	-	-	-	-	-	-	+	/	/	+	+	86	+	-	50	+	+	+	-	+	+	+	+	+	+	+	+	+	96	77	Unclear	Unclear	Unclear	Yes	Yes	Yes
Kubo et al., 2006a [37]	+	-	+	-	-	-	-	+	-	+	+	+	-	+	-	49	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	-	95	65	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Kubo et al., 2006b [71]	+	-	+	+	+	-	-	+	-	+	+	+	+	+	+	80	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	77	Unclear	Unclear	Unclear	Yes	Yes	Yes
Kubo et al., 2006c [72]	+	-	+	-	+	-	-	+	-	+	-	+	+	+	-	56	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	-	95	67	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Kubo et al., 2007 [55]	+	-	+	-	-	-	-	+	-	+	+	+	-	+	+	56	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	69	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
kubo [56]	+	-	+	-	+	-	-	+	-	+	-	+	+	+	-	56	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	-	95	67	Unclear'	Unclear'	Unclear	Yes	Yes	Yes
Kubo et al., 2010 [49]	+	-	+	+	-	-	-	+	-	+	+	+	+	+	+	78	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	76	Unclear	Unclear	Unclear	Yes	Yes	Yes
Kubo et al., 2012 [73]	+	-	+	+	-	-	-	+	-	+	+	+	+	+	-	70	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	73	Unclear	Unclear	Unclear	Yes	Yes	Yes
Malliaras et al., 2013 [38]	+	+	+	+	+	-	-	+	-	+	+	-	-	+	+	80	+	-	50	+	+	+	+	+	+	+	+	+	+	+	+	+	100	77	Unclear	Yes	Yes	Yes	Yes	Yes
Seynnes et al., 2009 [30]	+	+	+	-	+	-	-	+	+	-	-	+	+	+	+	78	+	-	50	+	+	+	-	+	+	+	+	+	+	+	+	+	96	74	Unclear'	Unclear'	Unclear	Yes	Yes	Yes

Methodological quality: 1 Study design | 1.1 Mechanical properties | 1.2 Material properties | 1.3 Morphological properties | 1.4 Control group **| 2 Methods | 2.1 Mechanical properties** | 2.1A Object of investigation | 2.1B Gravitational forces | 2.1C Axes misalignment | 2.1D Antagonistic muscle activation | 2.1E Lever arm measured | 2.1F Joint angle change | 2.1G Used multiple trials **| 2.2 Morphological properties** |2.2A MRI | 2.2B different positions **| 3 Cofactors** | 3A Gender | 3B Activity level **| 4 Statistical tests | 5 Power analysis | 6 Eligibility** | 6.1 Participants | 6.2 Variables **| 7 Description exercise protocol** | 7A Intensity | 7B Duration single stimulus | 7C Repetitions | 7D Sets | 7E Weeks | 7F times per week | **8 Description participants |** 8A Gender| 8B Age | 8C Body height | 8D Body weight | 8E Activity level; The single criteria were rated ("+" = point, "-" = no point, "/" = not included) and used to calculate the quality score for each category (i.e. internal, statistical and external validity). The average of the three scores gives the total score. A white head of the table box indicates that a full point was assigned to each sub-category for the calculation of the score in the respective validity section (assigned points / possible points*100), whereas a grey head of the table box indicates that the sub-categories of the respective block were pooled to a single point (assigned points / possible points).

Risk of bias [43]**: Sequence:** Adequate sequence generation, Allocation: Allocation concealment, Blinding: Blinding outcome assessor, Outcome: Incomplete outcome data, Report: Selective outcome reporting, Other: Other sources of bias; **Judgment:** Yes: low risk of bias, Unclear: insufficient information reported (': only one group, ⁺: Significant difference of baseline tendon cross-sectional area values between control and training group).

The three studies of Foure et al. [35,52,53] and the two studies of Kubo et al. [34,39] were merged as one, since the results of one intervention were reported in different publications.

Study	Stiffness				Young’s modulus				CSA			
	SMD	-CI	+CI	W	SMD	-CI	+CI	W	SMD	-CI	+CI	W
Albracht et al., 2013 [15]	0.82	0.02	1.63	3.8%								
Arampatzis et al., 2007 [29]	0.36	-0.49	1.20	3.6%	-0.05	-0.88	0.79	8.5%	0.23	-0.61	1.07	3.9%
	1.52	0.55	2.49	3.0%	0.75	-0.12	1.62	8.3%	0.64	-0.22	1.50	3.8%
Arampatzis et al., 2010 [28]	-0.25	-1.09	0.59	3.6%	-0.19	-1.03	0.64	8.5%	0.08	-0.75	0.92	3.9%
	0.79	-0.09	1.66	3.4%	0.56	-0.30	1.41	8.4%	0.00	-0.84	0.84	3.9%
Carroll et al., 2011 [57]	0.99	-0.14	2.13	2.4%	1.42	0.20	2.64	6.3%	-0.19	-1.03	0.65	3.9%
Fletcher et al., 2010 [17]	0.66	-0.52	1.84	2.2%								
Fouré et al., 2009 [61]	0.12	-1.01	1.26	2.4%								
Foure et al., 2010a, b, 2011 [35,52,53]	0.48	-0.46	1.42	3.1%					0.08	-0.85	1.00	3.4%
Foure et al., 2013 [40]	0.39	-0.45	1.24	3.5%					-0.11	-0.95	0.73	3.9%
Hansen et al., 2003 [58]	0.63	-0.23	1.49	3.5%					0.00	-0.84	0.84	3.9%
Hougthon et al., 2013 [36]	-0.15	-1.20	0.90	2.7%	-0.36	-1.42	0.70	7.2%	1.12	-0.04	2.28	2.4%
Kongsgaard et al., 2007 [24]	1.33	0.43	2.22	3.3%	1.05	0.19	1.92	8.4%	0.33	-0.48	1.13	4.1%
	-0.8	-1.64	0.03	3.6%	-0.29	-1.09	0.52	8.7%	0.46	-0.36	1.27	4.1%
Kubo et al., 2001a, b [34,39]	0.54	-0.47	1.54	2.8%					0.16	-0.82	1.14	3.1%
	1.31	0.20	2.42	2.5%	4.45	2.42	6.47	3.4%	0.15	-0.84	1.13	3.1%
Kubo et al., 2002 [25]	0.8	-0.23	1.83	2.7%					-0.22	-1.21	0.76	3.1%
	0.94	-0.11	1.99	2.7%					0.21	-0.78	1.19	3.1%
Kubo et al., 2003 [59]	0.42	-0.43	1.27	3.5%								
Kubo et al., 2006a [37]	0.25	-0.68	1.17	3.1%					0.14	-0.79	1.06	3.4%
	1.17	0.15	2.18	2.8%					0.19	-0.74	1.12	3.4%
Kubo et al., 2006b [71]	-0.01	-0.99	0.97	2.9%					0.00	-0.98	0.98	3.1%
Kubo et al., 2006c [72]	0.2	-0.73	1.13	3.2%					0.00	-0.92	0.92	3.4%
Kubo et al., 2007 [55]	0.51	-0.38	1.41	3.3%					0.21	-0.67	1.09	3.6%
	1.01	0.07	1.96	3.1%					-0.12	-1.00	0.76	3.6%
Kubo et al., 2009 [56]	0.94	0.01	1.88	3.1%					0.25	-0.63	1.14	3.6%
	0.58	-0.32	1.48	3.3%					0.13	-0.75	1.01	3.6%
Kubo et al., 2010 [49]	1.12	0.04	2.20	2.6%					0.05	-0.93	1.03	3.1%
Kubo et al., 2012 [73]	3.17	1.68	4.66	1.5%					0.23	-0.70	1.16	3.4%
Malliaras et al., 2013 [38]	1.03	0.03	2.03	2.8%	1.22	0.19	2.24	7.4%	0.62	-0.33	1.57	3.2%
	0.78	-0.13	1.70	3.2%	0.75	-0.17	1.66	8.0%	0.32	-0.56	1.21	3.6%
	1.35	0.36	2.34	2.9%	1.25	0.28	2.23	7.7%	0.35	-0.53	1.24	3.6%
Seynnes et al., 2009 [30]	0.81	0.06	1.56	4.1%	0.57	-0.16	1.31	9.2%	3.89	2.62	5.17	2.1%
Weighted average effect size	0.66	0.46	0.87		0.66	0.23	1.10		0.24	0.04	0.45	
Test for overall effect	P < 0.00001				P = 0.003				P = 0.02			
Heterogeneity	P = 0.04	I^2= 33%			P < 0.0008	I^2= 64%			P = 0.06	I^2= 31%		

Forest plot

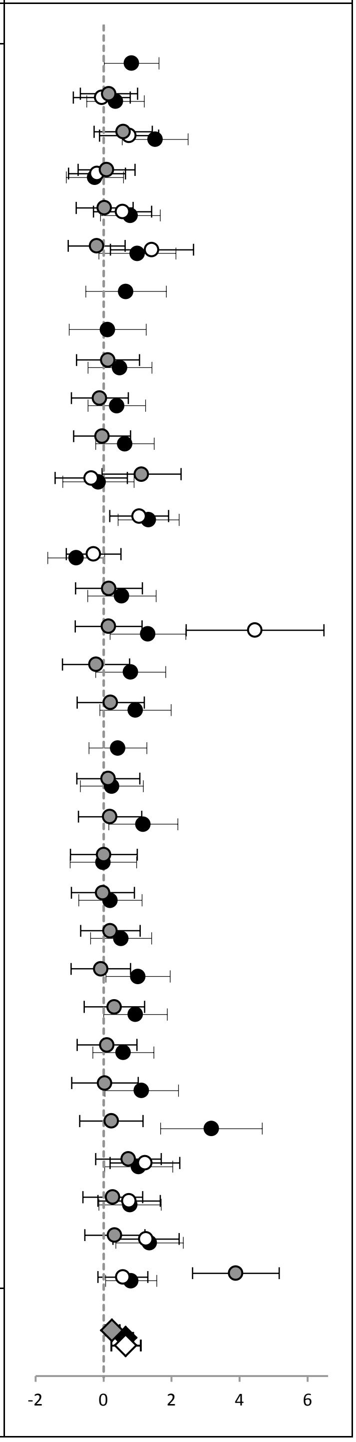

Fig. 4.2 Forest plot for the meta-analysis of the effect of mechanical loading (i.e. exercise interventions) on tendon stiffness (black), Young’s modulus (white) and cross-sectional area (CSA, grey), respectively, featuring the single study effect sizes (SMD, circles), the corresponding confidence intervals (CI, error bars) and study weight in the overall comparison (W) as well as the respective weighted average effect sizes (random-effect model, diamonds) with the overall effect test and heterogeneity analysis.

4.5 Discussion

The present meta-analysis assessed the effect of chronic mechanical loading on the adaptive responses of tendon mechanical (stiffness), material (Young's modulus) and morphological (cross-sectional area (CSA)) properties reported in the recent literature. Twenty-six studies, which provided an overall number of 33 separate exercise interventions (participants in total n=317), were included in the analysis. The weighted averaged effect size of the intervention-induced adaptations was 0.66 for tendon stiffness (n=33), 0.66 for Young's modulus (n=13) and 0.24 for CSA (n=29), indicating a moderate to large effect for the first two parameters and a small to moderate effect for the latter. The overall intervention effect for stiffness, Young's modulus and CSA was significant, regardless of the variety of applied loading regimens. However, the significant heterogeneity of stiffness and Young's modulus and tendency towards heterogeneity for CSA between the included interventions indicated that the different levels of the loading conditions might affect the adaptive responses. This meta-analysis gives further evidence for the plasticity of human tendon mechanical, material and morphological properties in vivo in response to chronic loading of various types. Moreover, the analysis showed that the adaptive response of the tendon to intervention-induced chronic loading might be more pronounced for the material compared to morphological properties.

The averaged effect size of the intervention-based changes of tendon stiffness was 0.66, featuring a significant overall effect of all included exercise interventions. Out of the 33 interventions that measured tendon stiffness, 22 showed effect sizes above 0.5 (i.e. medium to large effects [60]). Therefore, the present meta-analysis emphasizes the adaptive potential of tendons to increased mechanical loading, which was quite consistently shown despite the marked variety of loading protocols. However, the significant heterogeneity of tendon stiffness changes between studies indicated that especially the different levels of the applied loading conditions (e.g. intensity, duration, repetitions, sets, intervention duration and training frequency per week) and general exercise conditions (e.g. type of muscle contraction (isometric, concentric or eccentric) applied repetitively or statically, differences in joint angles that affect the tendon lever arm length and, thus, acting stress on the tendon) may considerably affect tendon adaptive responses. Indeed, this diversity of the applied loading protocols did not allow a further subgroup analysis and meta-regression. Furthermore, a consistent methodological approach to define and control the mechanical load on the tendon does to date not exist, which compromises the comparability of the study outcomes. However, several studies modified single loading parameters in their interventions to assess the respective effects on tendon adaptation, providing notable results. Arampatzis et al. [28,29], Kongsgaard et al. [24] and Malliaras et al. [38] investigated the effect of the magnitude of the mechanical load by means of low and high muscle contraction intensities. The studies reported a significant increase of tendon stiffness solely following the training using the high contraction intensities (i.e. 90% MVC, 70% one repetition maximum (RM), 80% eccentric RM, respectively). Pooling interventions using muscle contraction intensities higher than 70% of MVC or RM (n=23) and those using lower intensities (n=5) resulted in a weighted averaged effect size of tendon stiffness of 0.89 (CI 0.69, 1.09) and 0.04 (CI -0.46, 0.53), respectively, without heterogeneity (p=0.46, I2=0%) between studies using high intensities. Therefore, the present meta-analysis verifies the importance of high tendon loading as an appropriate stimulus for tendon adaptation. Considering the high contraction intensity studies, the present meta-analysis further indicates that the additional effect of the type of muscle contraction (isometric, concentric and eccentric) was minor (tab. 2). When separating the interventions using a high intensity in regard to the type of muscle contraction, the weighted averaged effect sizes of tendon stiffness showed comparable values between the isometric (n=12, SMD=0.93, CI 0.56, 1.29) versus the dynamic (n=11, SMD=0.88, CI 0.60, 1.16) training type. Therefore, we can argue that the level of tendon loading determines the stimulus for tendon adaptation independent of the muscle contraction type, which may explain the lack of differences between the interventions using different muscle contraction types. However, several of the included studies evidenced that beside the magnitude of tendon loading additional loading and exercise conditions may affect tendon adaptation, e.g. loading frequency [28], joint angle [37], loading duration [34,39] and repetitive vs. static loading [56]. Furthermore, the effect of plyometric training on tendon properties seems yet unclear, since the four plyometric training interventions [35,52,53,55,61] included in the present meta-analysis reported controversial results. Whereas Foure et al. [35,52,53] and Kubo et al. [55] reported an increase of tendon stiffness of 27% (statistically significant) and 19% (statistically not significant), respectively, the results of Fourè et al. [61] showed only small changes of 4% and of Houghton et al. [36] even a decrease of 9%. The different jumping exercises, uncontrolled [35,36,40,52,61] or comparably low (40% RM [55]) tendon load magnitude and dissimilar intervention durations (8-14 weeks) might be the reason for the inhomogeneous findings. In regard to the duration of the exercise intervention, several of the included studies featuring a duration of 8 weeks found significant adaptations of tendon stiffness [17,25,30], indicating that tendons already respond to increased mechanical loading within two months. Pooling the interventions featuring a high intensity with respect to the intervention duration, the weighted average effect sizes of tendon stiffness were 0.92 for the interventions using longer durations (≥12 weeks: n=19, g=0.92, CI 0.67, 1.16) and 0.81 for the shorter ones (8-12 weeks: n=4, g=0.81, CI 0.33, 1.29). The present analysis showed that shorter intervention durations (8-12 weeks) may induce tendon adaptive responses, however, longer durations (≥12 weeks) seem to be more efficient and their effect has been clearly demonstrated in many studies. The present meta-analysis solely included data of Achilles and patellar tendons. However, as to be expected, similar loading protocols on different types of tendons induced similar adaptive responses [54].

Therefore, evidence-based interventions that facilitate tendon adaptation should be applicable to various tendons and prove valuable in regard to athletic training as well as the therapy and prevention of tendon injuries.

Increases in tendon stiffness may be a result of either change in tendon material properties (i.e. Young's modulus) and/or tendon morphological properties (i.e. cross-sectional area and tendon rest length). Several studies reported increases of tendon CSA following training interventions [24,28–30]. However, it is feasible that no such reports exist for an exercise-induced change of tendon rest length, which hence can be excluded from being a relevant adaptive mechanism in response to increased mechanical loading. Regardless of the differences between the applied loading regimens, the averaged effect size for Young's modulus (n=13) was 0.66 and for CSA 0.24 (n=29). The overall intervention effect was significant for both, Young's modulus and CSA and the heterogeneity between studies was significant for Young's modulus and in tendency for CSA. As averaged effect size of stiffness and Young's modulus were similar and comparably higher as the CSA effect size, we can argue that the increase in stiffness may be primarily attributed to alterations of the material properties rather than morphological properties. Changes of the material properties were mentioned to be an early mechanism for increased stiffness, whereas tendon hypertrophy could be a long-term effect of mechanical loading [19,31]. Several studies included in the present meta-analysis found an increase in tendon Young's modulus following the exercise interventions without changes in the tendon CSA [26,28,39], supporting the assumption that material properties demonstrate greater plasticity and change more instantaneous in response to enhanced chronic mechanical loading. Taking into account that the average duration of all included interventions was 12.8 ± 4.7 weeks (two studies with longer durations than 14 weeks: running training [58] and low load resistance bodyweight training [59]), the reason for the small effect size of CSA in contrast to the larger effects of Young's modulus may be the relatively short intervention durations. Yet, tendon hypertrophy could be more pronounced following longer periods of loading (i.e. habitual loading) compared to durations commonly used in exercise interventions.

The appropriate investigation of tendon properties needs to include numerous methodological considerations. The total methodological quality score used in the present meta-analysis ranged from 61-92% with a mean of 70 ± 8%, indicating adequate to high methodological qualities for most studies and thus, study validity. However, several aspects of the internal study validity (i.e. study design, methods and co-factors) were not considered in every study. First, only 13 of the 33 included interventions reported the values of stiffness, Young's modulus and CSA and, therewith, provided a complete examination of the adaptive processes of the mechanical, material and morphological tendon properties and their interaction. Less than half (i.e. 15) of the 33 interventions included a control group. During the measurement and calculation of the tendon force, tendon elongation and CSA, which are necessary to assess the tendon properties, no study considered all relevant methodological aspects (e.g. accounting for gravitational forces, axes misalignment of joint and dynamometer, averaging multiple trials to reliably assess tendon elongation, tendon arm directly measured), which affects the validity of the applied method. In consequence, the score for the internal validity was in mean only 64 ±14% (range: 49-91%). In regard to the statistical validity all studies applied appropriate statistical tests, but only one study [36] calculated the effect size to estimate the effect of the intervention-induced tendon adaptations. Furthermore, care was not always taken in controlling and reporting all relevant loading conditions (e.g. intensity, duration of loading) [35,36,40,52,61], compromising the comparability of the results between interventions and their interpretations in regard to potential causalities. Nevertheless, a mean external validity score of 96 ± 4% (range 88-100%) indicated a high external validity of all included studies. Although already considered in most present studies, future investigations on tendon adaptation should account for these methodological quality criteria to ensure high study validity. The risk of bias assessment was difficult, since important information were not reported in most articles. In particular, details of the randomization process, concealment of allocation and/or blinding of the assessor to the outcome data were missing in 30 of the 33 interventions and, therefore, the risk of bias judgment was inadequate for most included interventions. Only three studies [24,38,57] provided the necessary information and the assessment indicated a low risk of bias. However, the judgment of the other domains (i.e. incomplete outcome data, selective outcome reporting and other sources of bias) indicated a low risk of bias for almost every included study. Future investigations should account for an appropriate consideration and/or presentation of these aspects to allow for risk of bias estimation. Although risk of bias assessment could partly not performed adequately due to a lack of information reporting, the overall assessment together with the methodological quality scale indicated an appropriate validity of the included studies and, thus, the outcome of the present meta-analysis.

The current review and meta-analysis may feature some limitations in regard to the sample sizes, recruited participants and durations of the included interventions. All included studies were performed on small sample sizes (6-15 participants), most likely due to the great study effort, and, thus, conclusions with regard to a greater population based on solely one intervention should be drawn carefully. However, the present meta-analysis on recent literature confirmed the effects of chronic loading on the adaptation of mechanical, material and morphological tendon properties. To a greater part the included participants were male (291 of 317) and involved in recreational activity (approximately 255 of 317), which could have biased the generalizability of the study outcomes to a greater mixed-gender population with a different activity profile. Furthermore, the duration of 31 of the 33 included interventions was short-term (≤14 weeks). However, longer durations may affect the adaptive responses of the separate tendon properties (material and morphological) in a different way.

4.6 Conclusion

In conclusion, the present meta-analysis on the effect of chronic mechanical loading on human tendon adaptation in vivo included 26 studies featuring 33 separate exercise interventions. The meta-analysis showed that tendons are highly responsive to increased mechanical loading and adapt through changes of their mechanical, material and morphological properties. Intervention-induced changes in tendon stiffness seem to be more attributed to adaptations of the material rather than morphological properties. Although a lot of research of the past decade contributed to a deeper understanding of tendon adaptation, several aspects remain unclear (e.g. effects of loading duration and rate of loading) and need further clarification.

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5. Third study:

Human Achilles Tendon Plasticity in Response to Cyclic Strain: Effect of Rate and Duration

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5.1 Abstract

High strain magnitude and low strain frequency are important stimuli for tendon adaptation. Increasing the rate and duration of the applied strain may enhance the adaptive responses. Therefore, our purpose was to investigate the effect of strain rate and duration on Achilles tendon adaptation.

The study included two experimental groups (n=14 and n=12) and a control group (n=13). The participants of the experimental groups exercised (14 weeks, 4x/week) according to a reference protocol, featuring a high strain magnitude (~6.5%) and a low strain frequency (0.17 Hz, 3 s loading/3 s relaxation) on one leg and with either a higher strain rate (one-legged jumps) or a longer strain duration (12 s loading) on the other leg. The strain magnitude and loading volume were similar in all protocols. Before and after the interventions the tendon stiffness, Young's modulus and cross-sectional area were examined using magnetic resonance imaging, ultrasound and dynamometry.

The reference and long strain duration protocols induced significantly increased ($p<0.05$) tendon stiffness (57% vs. 25%), cross-sectional area (4.2% vs. 5.3%) and Young's modulus (51% vs. 17%). The increases in tendon stiffness and Young's modulus were higher in the reference protocol. Although region-specific tendon hypertrophy was also detected after the high strain rate training, there was only a tendency of increased stiffness ($p=0.08$) and Young's modulus ($p=0.09$). The control group did not show any changes ($p>0.05$).

The results provide evidence that a high strain magnitude, an appropriate strain duration and repetitive loading are essential components for an efficient adaptive stimulus for tendons.

Keywords:

Exercise, load, MRI, tendon adaptation, tendon training, tendon hypertrophy

5.2 Introduction

Tendinous tissue is highly sensitive to mechanical loading. The external strain of the tendon following contractions of the attached muscle is transmitted through the extracellular matrix on the cytoskeleton of the mechanosensitive tendon cells via membrane proteins (e.g. integrins, G-protein, receptor and protein kinases; Wang, 2006). The deformation of tendon cells initiates the expression of genes responsible for catabolic and/or anabolic cellular and molecular responses (e.g. collagen synthesis), which affect the mechanical (stiffness), morphological (cross-sectional area) and material (Young's modulus) tendon properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011b; Kjaer, 2004; Lavagnino and Arnoczky, 2005; Wang, 2006). From a mechanobiological point of view, four conditions in the applied strain may affect the adaptive response of tendons: magnitude, frequency, rate and duration (Arnoczky et al., 2002a; Lavagnino et al., 2008; Yamamoto et al., 2005, 2003; Yang et al., 2004). Recent experiments on the human Achilles tendon (AT) in vivo by our group showed that a high strain magnitude (4.5 - 5.0%) is required to trigger adaptive effects of the tendon mechanical, morphological and material properties (Arampatzis et al., 2010, 2007a). Furthermore, we found that applying the same high strain magnitude with a low strain frequency (i.e., 0.17 Hz, 3 s loading/3 s relaxation vs. 0.5 Hz, 1 s loading/1 s relaxation) leads to superior adaptive responses of the tendon properties (Arampatzis et al., 2010). However, the possibility of a superimposed effect of the applied tendon strain rate and tendon strain duration in relation to a high strain magnitude and a low strain frequency on the plasticity of the mechanical, morphological and material tendon properties of humans in vivo has not been investigated yet. A profound understanding of tendon plasticity is essential, particularly with regard to tendon adaptation and tendon healing. Such information could allow for improvements in human locomotor performance, as well as tendon injury prevention and rehabilitation.

The cellular adaptive responses of tendons are dependent on the transmission of the external tendon strain via the extracellular matrix to the mechanosensitive tendon cells. The strain on the cellular level is much lower compared to the external tendon strain (Arnoczky et al., 2002a; Screen et al., 2005). Two modes were suggested for the transmission of the external strain to the cellular level: cell deformation and fluid flow-induced shear stress (Lavagnino et al., 2003, 2008). With increasing strain, a loss of collagen crimp and an increase in fiber recruitment was observed (Hansen et al., 2002; Schatzmann et al., 1998) and likely result in an increased number of cells being deformed (Arnoczky et al., 2002b). The inhibition of catabolic cell responses (collagenase mRNA) seems to be directly associated with progressive increases of strain magnitude (Arnoczky et al., 2004; Lavagnino et al., 2008, 2003). Furthermore, the extracellular matrix features viscous behavior due to the content of collagen, water and interactions between collagenous and non-collagenous properties (Ciarletta and Amar, 2009; Wang, 2006), and, thus, a time-dependent transmission of the external tendon strain to the cytoskeleton. Therefore, we can argue that longer durations of tendon strain may more effectively transmit external strain on the cells and likely result in a greater cell deformation, thus, providing a superior adaptive stimulus compared to shorter ones.

In addition to cell deformation, fluid flow-induced shear stress (hydrostatic stress) was suggested to be another stimulus that affects tendon adaptation (Archambault et al., 2002; Giori et al., 1993; Lavagnino et al., 2008, 2003). In vitro experiments demonstrated the existence of interstitial fluid flow within the tendinous tissue following cyclic loading (Hannafin and Arnoczky, 1994; Helmer et al., 2006; Lanir et al., 1988). Load-related fluid flow was shown to induce a shear stress on the cell membrane, which stimulated the tendon cells to alter their gene expression (Archambault et al., 2002). Moreover, the applied tendon strain rate may determine the fluid flow-induced shear stress (Haut and Haut, 1997; Lavagnino et al., 2008). Lavagnino et al. (2008) reported that an increase in strain rate from 2%/min to 20%/min using the same strain magnitude decreased the interstitial collagenase mRNA expression in rat tail tendon significantly (relative quantification of MMP-13: 1401 versus 33, respectively), indicating that strain rate-mediated fluid shear stress alone is efficient to inhibit catabolic gene expression. Therefore, increased strain rate may enhance the adaptive response of human tendons in vivo.

From a mechanobiological point of view we can argue that tendon adaptation is a result of the close interaction of the mechanical environment and the biology of the tendinous tissue. In regard to this interaction it is of great importance to understand the mechanical conditions (e.g. strain rate-mediated fluid shear stress and time-dependent cell deformation) that may influence the tendon adaptive responses in vivo, not only in applied but also in basic research. Hence, our previous (Arampatzis et al., 2010, 2007a) and present experiments concentrated on the identification of the most appropriate mechanics for biological responses (i.e., tendon adaptation). The purpose of the present study was to investigate the potential of a superimposed effect of strain rate and strain duration applied to the tendon compared to high strain magnitude and low strain frequency on the adaptation of the mechanical, morphological and material properties of the human AT in vivo. The strain rate and duration were modified with respect to a reference exercise protocol (i.e., high tendon strain magnitude and low strain frequency), which induced the most superior adaptive effects in our earlier studies (Arampatzis et al., 2010, 2007a). Because isometric muscle contractions as in our previous studies (Arampatzis et al., 2007, 2010) did not allow to increase the strain rate adequately, the higher strain rate was achieved by means of impact loading (i.e., jumping). Based on the expected time-dependency of the transfer from the external strain to the cellular level, we hypothesized that a longer tendon strain duration compared to the reference protocol would facilitate the adaptation of the mechanical, morphological and material tendon properties. Further, we hypothesized that a higher strain rate compared to the reference protocol may provide an additional adaptive stimulus and enhance the adaptive responses of the tendon, most likely due to an associated increase of hydrostatic pressure and fluid flow-induced cell shear stress as suggested from in vitro studies.

5.3 Methods

5.3.1 Subjects

Thirty-nine male adults participated in the present study after giving informed consent to the experimental procedure, which was approved by the local ethics committee. The participants were randomly assigned either to one of the two experimental groups (group 1: n = 14, age 26.7 ± 4.2 yr, weight 82.2 ± 13.1 kg, height 182.3 ± 5.3 cm; group 2: n = 12, age 29.5 ± 3 yr, weight 74.8 ± 7.3 kg, height 177.6 ± 7 cm) or to a control group that received no specific training (n = 13, age: 26.5 ± 4.5 yr, weight: 78.6 ± 10.7 kg, height: 182.3 ± 10.7 cm). All participants reported no musculoskeletal impairments of the lower limbs and were physically active but not involved in high-performance sports.

A statistical power analysis was performed a priori using the AT stiffness and Young's modulus values (n = 11) from the most effective exercise protocol of our previous intervention experiments (Arampatzis et al., 2010, 2007) to calculate the required sample size for an appropriate statistical power. The analysis (α = 0.05, power = 0.95, correlation = 0, Effect size: stiffness 1.6, Young's modulus 1.2) by means of the software G*Power (version 3.1.9.2, Germany) revealed that a sample size of n = 12 would be sufficient to achieve a high statistical power (i.e., 0.95) of the expected outcome.

5.3.2 Exercise interventions

To investigate the effect of the strain rate and strain duration on tendon adaptation, two separate exercise interventions were conducted for 14 weeks with 4 sessions per week. Following a standardized warm-up, 5 sets of plantar flexion contractions were used to induce cyclic strains of the AT. In both interventions one randomly assigned leg of each participant was exercised following a reference protocol similar to the one that induced the most superior adaptive effects of the AT mechanical and morphological properties in our earlier studies (Arampatzis et al., 2010, 2007). The reference protocol included repetitive isometric contractions (4 times 3 s loading, 3 s relaxation, fig. 5.1) on a leg press (ankle angle 5° dorsal flexion, knee joint fully extended and hip flexed at 115°). In the first intervention (group 1) the contralateral leg was trained by means of one-legged jumps (72 per set), thus, increasing the strain rate with respect to the reference protocol (high strain rate protocol, fig. 5.1). The short contact phases during the jumping were associated with a ~3 times faster force development compared to the isometric contractions of the reference protocol (time to peak force was ~130 ms and during the reference isometric contractions ~380 ms), indicating a higher strain rate of the AT compared to the reference protocol. With regard to our earlier findings (Arampatzis et al., 2010, 2007) we set the target force in both, reference and high strain rate protocol, to 90% of the maximal voluntary isometric plantar flexion force (MVC) to induce a high strain magnitude on the AT, i.e. 6.63 ± 1.24% and 6.43 ± 1.18% (mean of pre and post measurements ± SD), respectively. The MVC target values were updated every 10 training sessions. A pilot study was conducted to compare the ankle joint moments during the one-legged jumps with the isometric condition and the results indicated similar joint moments and, thus, magnitude of tendon loading during both conditions. The loading volume (integral of plantar flexion force over time) was kept similar in both protocols by adjusting the number of contractions (i.e., 4 times 3 s loading and 3 s relaxation in the reference protocol and 72 jumps in the high strain rate protocol) to ensure direct comparability (fig. 5.1). Visual feedback of the exerted and target plantar flexion force was given to the participants during the training (fig. 5.1). Before starting both interventions two weeks of reduced low intensity exercise were performed to accustom the participants to the specific load.

The second intervention (group 2) aimed to investigate the effect of a modification of the strain duration on the tendon adaptation. On one leg the participants exercised the same reference protocol as applied during intervention 1 (repetitive isometric contractions, 4 times 3 s loading, 3 s relaxation per set). In contrast, the other leg performed a single 12 s isometric plantar flexion contraction per set and, therefore, the strain duration applied on the AT was 4 times longer compared to the reference protocol (fig. 5.1). Both reference and long stain duration protocol included the high strain magnitude (i.e., 6.49 ± 1.49% and 6.94 ± 1.54% (mean of pre and post measurements ± SD), respectively) and loading volume of the first intervention (i.e., 90% of the MVC). By means of this experimental design that featured the same magnitude and volume of loading in both interventions (i.e., strain rate and strain duration) we were able to compare directly the effects of strain rate and strain duration modulation on the AT adaptation. Before and after the interventions the mechanical, morphological and material properties of the AT of both legs were assessed. For the control group the AT mechanical properties were measured at the left and right leg and both values were included in the further analysis.

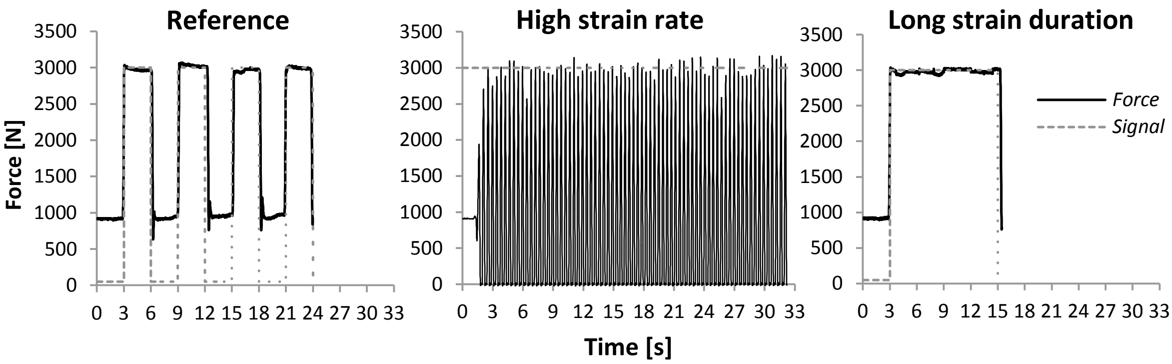

Fig. 5.1 Loading profiles of the reference protocol (4 repetitions of 3 s loading, 3 s relaxation), the high strain rate protocol (72 one-legged jumps) and the long strain duration protocol (1 repetition of 12 s loading) of the two interventions performed for 5 sets on 4 days per week for 14 weeks, featuring similar exercise volume (integral of the plantar flexion force over time). The rate was increased by factor ~ 3 and duration by factor 4 with respect to the reference protocol to investigate their effect on the Achilles tendon (AT) adaptation. Plantar flexion contractions at 90 % MVC were used to induce high magnitude strain of the AT. Signal: signal displayed to the participants to control the magnitude and volume of loading (i.e., time and individual target force). Force: plantar flexion force over time exerted during the exercising exemplarily for one participant.

5.3.3 Achilles tendon morphological properties

The morphological properties of the free AT (i.e., length and cross-sectional area (CSA)) were determined by means of magnetic resonance (MR) imaging. Only the participants of the two experimental groups (intervention 1 and 2) were investigated, since changes of the AT morphological properties of the control group participants without a specific stimulus were not expected (Kubo et al., 2010, 2006). A 0.25 T MR scanner (G-Scan, Esaote, Italy) captured transversal and sagittal MR scans of the AT (3D HYCE (GR) sequence, TR 10 ms, TE 5 ms, flip angle 80°, slice thickness 3 mm, 1 excitation) while the participants lay in supine position with the hip and knee extended and the ankle fixed in relaxed position. The sagittal images were used to detect the borders of the free AT, i.e. M. soleus-AT junction and initial attachment on the calcaneus bone, respectively (fig. 5.2). Every transversal slice within these borders was segmented manually using the software OsiriX (Pixmeo SARL, version 2.5.1., Switzerland) in order to determine the CSA of the tendon (fig. 5.2). Three independent observers analyzed the MR images of both legs from all participants and the mean values from all observers were used for the further analysis. The free AT length was calculated as the curved path through the centroids of the CSAs, which were calculated using a Delaunay triangulation (fig. 5.2). The CSA was displayed for 10% intervals along the free AT length to consider region specific changes of the tendon (Arampatzis et al., 2007). The average CSA of the AT has been calculated as the mean from all determined CSAs along the AT length.

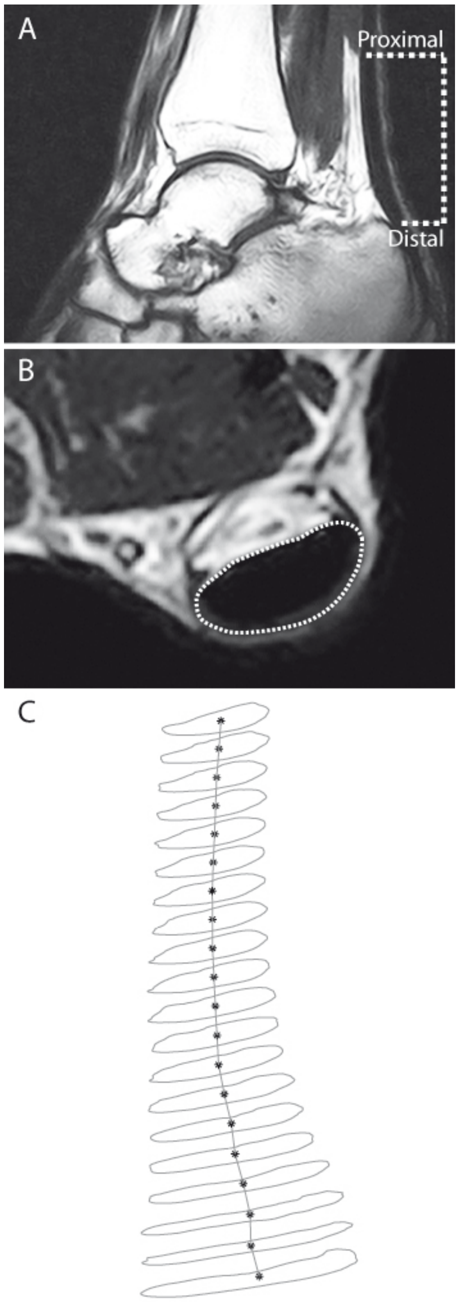

Fig. 5.2 Sagittal (A) and transverse (B) magnetic resonance images of free Achilles tendon (AT) were used to investigate the morphological AT properties (i.e., length and cross-sectional area (CSA), C). Transversal images served to detect the proximal (M. soleus-AT junction) and distal (initial attachment on the calcaneus bone) border of free AT (A). Sagittal images within this range were segmented to determine the CSA (B) and to calculate the tendon length (i.e., curved path through the CSA centroids, C).

5.3.4 Achilles tendon mechanical and material properties

The stiffness and Young's modulus of the AT were examined combining dynamometry, MRI and ultrasound measurements. The Achilles tendon force was calculated from the ankle joint moment and the tendon lever arm. Thereto, the participants performed maximal isometric plantar flexion contractions in seated position with the knee extended and the ankle angle at neutral position (tibia perpendicular to the sole of the foot, 90°) on a dynamometer (Biodex-System 3, Biodex Medical Systems Inc., USA). To account for axis misalignments of the ankle joint and dynamometer during the MVCs, resultant joint moments were calculated by means of inverse dynamics (Arampatzis et al., 2005). The relevant kinematic data were captured by an infrared motion capture system (Vicon Nexus, version 1.7.1., Vicon Motion Systems, UK) integrating 9 cameras operating at 250 Hz. Furthermore, the contribution of the antagonistic muscle tibialis anterior to the measured ankle joint moments was taken into account (Mademli et al., 2004). The activity of the tibialis anterior muscle during the maximum plantar flexions was recorded by means of electromyography (EMG; Myon m320RX, Myon AG, Switzerland). Based on the relationship of EMG amplitude of the M. tibialis anterior and the exerted moments during sub-maximal isometric dorsal flexion contractions, the corresponding antagonistic moment during the maximal plantar flexion could be calculated (Mademli et al., 2004). The AT force was calculated by dividing the ankle joint moment by the AT lever arm which was determined applying the tendon excursion method. This method is based on the ratio of the M. gastrocnemius medialis myo-tendinous junction (MTJ) displacement obtained by B-mode ultrasonography to the corresponding angular excursion of the ankle joint (An et al., 1984; Fath et al., 2010). Although this method not account for the tendon compliance, the magnitude of tendon elongation due to the ankle angle change was reported to be low in the range used for the lever arm calculation (i.e., 5 ° dorsal flexion to 10 ° plantar flexion; De Monte et al., 2006). Alterations of the tendon lever arm during the contraction were considered in the calculation using the factor suggested by Maganaris et al. (1998). The elongation of the AT during a ramped MVC (~ 5 s gradual increase of force) was measured by capturing the MTJ displacement using B-mode ultrasonography. A 10 cm linear probe (My Lab 60, Esaote, Italy) embedded in a custom-built foam cast was fixed to the shank and recorded the displacement of the MTJ at 25 Hz. Afterwards the displacement was traced manually frame by frame within a custom written MATLAB interface (The Mathworks, version 2012, USA). Displacements of the MTJ as a result of changes in the ankle joint angle during the MVC were subtracted, since they significantly affect the tendon elongation measurement (Arampatzis et al., 2008). For this purpose, the passive MTJ displacement in relation to the ankle angle was analyzed in an additional trial (inactive ankle rotated over the full range of motion of the ankle joint at 5 °/s). With regard to the reliability of ultrasound-based tendon elongation measurements reported by Schulze et al. (2012), we averaged the force and elongation data of five contractions.

The AT stiffness was calculated from the tendon force and tendon elongation ratio between 50 and 100% of the maximum tendon force using linear regression. The tendon rest length was measured from tuberositas calcanei to the MTJ at an ankle angle of 110° (plantar flexed) and extended knee, since in this position slackness of the inactive gastrocnemius medialis muscle-tendon unit has been reported (De Monte et al., 2006). The Young's modulus of the AT was calculated from the relationship of tendon stress and tendon strain from 50 to 100% of the maximum stress by means of linear regression. The AT stress was calculated as the quotient from the AT force and the averaged CSA and the AT strain as the quotient from the elongation and the rest length.

5.3.5 Statistics

Normal distribution of the data was examined using the Kolmogorov-Smirnov test. An analysis of variance for repeated measures (RM-ANOVA) was performed separately for both interventions in order to determine the effect of the intervention (within-subjects variable: before and after) as well as the effect of the different protocols (between-subjects factor: reference, high strain rate or long strain duration and control) on the AT stiffness. A Bonferroni post hoc analysis was conducted in the case of a significant interaction of the factors intervention and protocol. A similar RM-ANOVA was applied on the average CSA and Young's modulus values, however, the values of the control group were not present and, therefore, this group was not included in the between-subjects factor. The 10% intervals of the CSA along the AT length were tested for pre and post intervention differences using a paired t-test. The statistics were performed using the software SPSS Statistics (IBM, version 20, USA) and the level of significance for all statistical procedures was set at $\alpha = 0.05$. Furthermore, to estimate the strength of potential alterations of the investigated parameters following the interventions, the effect size (d) was calculated. Values of <0.20 indicate small, of 0.50 indicate medium, and >0.80 indicate large effects sizes (Cohen, 1988).

5.4 Results

5.4.1 Intervention 1: Effect of strain rate

The body mass of the participants of the intervention group 1 and the control group did not change during the 14 weeks of training (intervention: 82.2 ± 13 kg before training, 81.9 ± 13.5 kg after training; control: 78.6 ± 10.7 kg before, 78.3 ± 10.4 kg after).

There was a significant effect of the exercise intervention on the AT stiffness ($p < 0.05$) as well as an interaction of the factors intervention and protocol ($p < 0.05$). The stiffness increased significantly after the 14 weeks of exercise by the reference protocol ($p < 0.05$, fig. 5.3) while only a tendency towards higher values was found in the leg that was exercised using the high strain rate protocol ($p = 0.08$, fig. 5.3). The effect size of the AT stiffness increase in the reference and high strain rate protocol was $d = 1.08$ and $d = 0.69$, respectively. The stiffness values of the control group remain unchanged ($p > 0.1$, fig. 5.3). Regarding the 10% intervals of the AT cross-sectional area (CSA) along AT length, we found a significant increase in the proximal part from 40 to 100% tendon length ($p < 0.05$) in the leg that was exercised by means of the reference protocol (fig. 5.4). In the leg, trained by the high strain rate protocol we also found a significant region specific hypertrophy of the AT, but just in the 20-40%, 60-70% and 80-90% intervals ($p < 0.05$, fig. 5.4). Furthermore, there was a significant effect of the intervention on the average CSA of the AT ($p < 0.05$). The average CSA increased significantly after completing the training using the reference protocol ($p < 0.05$) and in tendency ($p = 0.09$) following the training using the high strain rate protocol (tab. 5.1). Further the Young's modulus of the AT increased significantly only in the reference protocol ($p < 0.05$, tab. 5.1). The length of the free AT did not change following the intervention with both protocols (tab. 5.1). The effect sizes of the investigated parameters with regard to the respective protocols are presented in table 5.1.

Tab. 5.1 Comparison of the investigated parameters before (Pre-exercise) and after (Post-exercise) intervention 1 featuring the reference and high strain rate protocol, respectively, and the respective effect sizes (d).

Parameter	Reference (n = 14)			High strain rate (n = 14)		
	Pre-exercise	*Post-exercise*	d	*Pre-exercise*	*Post-exercise*	d
CSA [mm^2]	79.9 ± 3.4	83.0 ± 3.7*	0.23	80.5 ± 3.9	82.5 ± 3.6	0.14
Young's modulus [GPa]	0.91 ± 0.07	1.43 ± 0.17*	1.08	0.92 ± 0.08	1.14 ± 0.13	0.55
Length [mm]	49.3 ± 5.4	49.7 ± 3.8	0.02	52.5 ± 5.8	52.9 ± 5.5	0.02

Note: The values present the mean ± standard error of mean of the average cross-sectional area (CSA) of the Achilles tendon (AT), Young's modulus of the AT and length of free AT (length).
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).

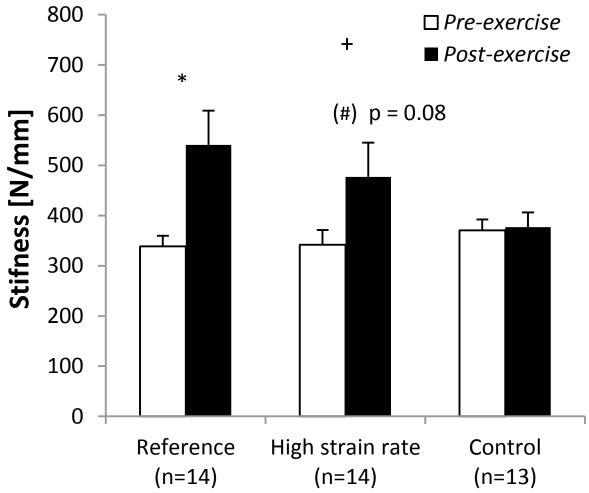

Fig. 5.3 Mean stiffness values and standard error of mean (error bars) of the Achilles tendon before (Pre-exercise) and after (Post-exercise) the intervention 1 featuring the reference and high strain rate protocol as well as for the control group.
+ : statistically significant interaction of intervention and protocol ($p < 0.05$). The post hoc comparisons show a significant increase only in reference protocol.
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).
(#) : tendency towards a difference to the Pre-exercise values ($p = 0.08$).

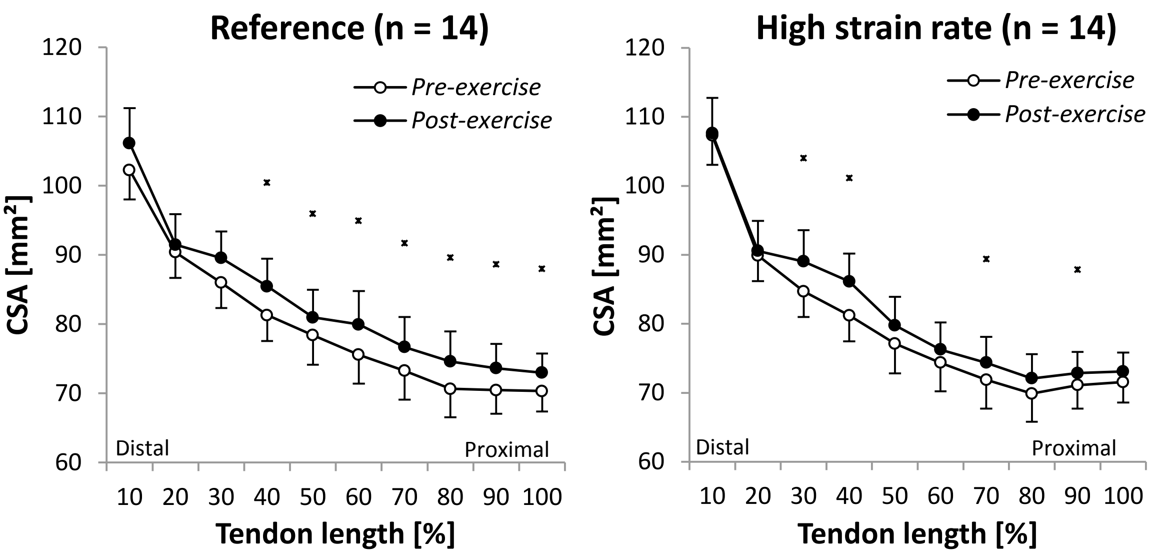

Fig. 5.4 Mean cross-sectional area (CSA) and standard error of mean (error bars) of the Achilles tendon in 10 % intervals of the tendon length before (Pre-exercise) and after (Post-exercise) intervention 1 featuring the reference and high strain rate protocol, respectively.
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).

5.4.2 Intervention 2: Effect of strain duration

The body mass of the participants of the second intervention group remained constant during the 14 weeks of training (74.8 ± 7.3 kg before training, 75 ± 7.2 kg after training).

The exercise intervention had a significant effect on the stiffness of the AT ($p < 0.05$). Further, there was an interaction between the factors intervention and protocol and the post hoc comparisons showed that the AT stiffness increased significantly ($p < 0.05$) following both training protocols (i.e., reference and long strain duration) but remain unchanged ($p > 0.1$) in the control group (fig. 5.5). Following the reference protocol, the increase in the AT stiffness was higher compared to the long strain duration protocol (fig. 5.5). The effect size of the stiffness increase following the reference and long strain duration protocol was d = 1.51 and 0.68, respectively. The 10% interval-analysis of the CSA along the tendon length showed a significant increase in the proximal part from 40 to 100% following the training with both, reference and long strain duration protocol ($p < 0.05$, fig. 5.6). Furthermore, the average CSA increased significantly ($p < 0.05$) following both protocols (tab. 2). There was a significant intervention effect on the Young's modulus of the AT ($p < 0.05$). The values increased significantly following the reference and long strain duration protocol training ($p < 0.05$, tab. 5.2). However, an interaction effect (intervention x protocol) indicated that the increase of the Young's modulus was more pronounced following the reference protocol (tab. 5.2). The length of the free AT did not change following the intervention with both protocols (tab. 5.2). The effect sizes of the investigated parameters in regard to the respective protocol of the second intervention are presented in table 5.2.

Tab. 5.2 Comparison of the investigated parameters before (Pre-exercise) and after (Post-exercise) intervention 2 featuring the reference and long strain duration protocol, respectively, and the respective effect sizes (d).

Parameter	Reference (n = 12)			Long strain duration (n = 12)		
	Pre-exercise	*Post-exercise*	d	*Pre-exercise*	*Post-exercise*	d
CSA [mm^2]	75.4 ± 2.6	78.8 ± 3.0*	0.35	78.1 ± 3.1	82.4 ± 3.6*	0.38
Young's modulus [GPa] #	0.97 ± 0.08	1.41 ± 0.11*	1.31	0.89 ± 0.08	1.05 ± 0.08*	0.57
Length [mm]	56.5 ± 3.9	56.0 ± 3.8	-0.03	54.2 ± 3.7	53.8 ± 3.8	-0.03

Note: The values present the mean ± standard error of mean of the average cross-sectional area (CSA) of the Achilles tendon (AT), Young's modulus (Y modulus) of the AT and length of free AT (length).
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).
# : statistically significant interaction (intervention x protocol) ($p < 0.05$).

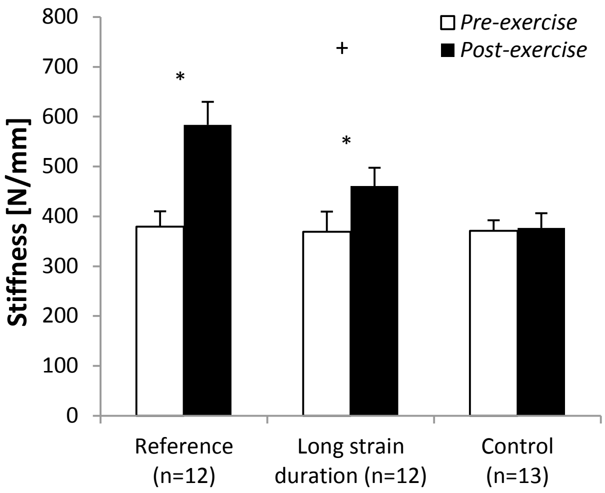

Fig. 5.5 Mean stiffness values and standard error of mean (error bars) of the Achilles tendon before (Pre-exercise) and after (Post-exercise) the intervention 2 featuring the reference and long strain duration protocol as well as for the control group.
+ : statistically significant interaction of intervention and protocol ($p < 0.05$) indicating an increase following both exercise protocols and a pronounced increase following the reference compared to the long strain duration protocol ($p < 0.05$).
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).

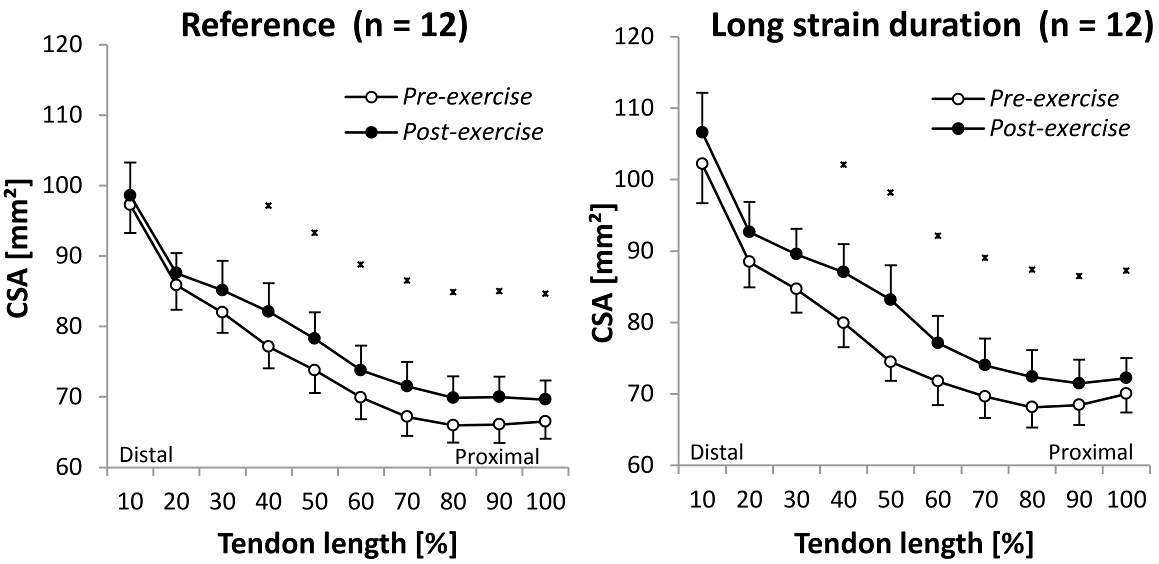

Fig. 5.6 Mean cross-sectional area (CSA) and standard error of mean (error bars) of the Achilles tendon in 10 % intervals of the tendon length before (Pre-exercise) and after (Post-exercise) intervention 2 featuring the reference and long strain duration protocol, respectively.
* : statistically significant difference from the Pre-exercise values ($p < 0.05$).

5.5 Discussion

The present study investigated the potential of a superimposed effect of strain rate and strain duration on the Achilles tendon (AT) adaptation of healthy young male adults and completes our earlier experiments that focused on the effects of strain magnitude and strain frequency (Arampatzis et al., 2010, 2007). Two 14-week interventions were conducted featuring a controlled modification of the strain rate and strain duration of the AT, respectively. The participants exercised a reference protocol similar to the one which induced the most superior adaptive effects in our earlier experiments on one leg and either a comparatively higher strain rate (intervention 1) or longer strain duration (intervention 2) on the other leg. After completing the training using the reference and the long strain duration protocols we found a clear increase of the AT stiffness, average CSA and Young's modulus. However, compared to the long strain duration protocol, the increase of AT stiffness and Young's modulus was more pronounced following the reference protocol. Although a region specific hypertrophy of the tendon was also detected following the high strain rate protocol, average CSA and AT stiffness showed only a tendency towards higher values and the Young's modulus did not change at all. Based on these findings we have to reject both hypotheses.

In our initial hypotheses we postulated that the increased hydrostatic pressure and fluid flow-induced shear stress within the tendinous tissue associated with a higher tendon strain rate (i.e., induced by one-legged jumps) (Giori et al., 1993; Haut and Haut, 1997; Helmer et al., 2006) would be an additional stimulus for tendon adaptation (Archambault et al., 2002; Giori et al., 1993; Lavagnino et al., 2008). However, the adaptive responses of the AT properties after the high strain rate protocol were lower compared to the reference one. Although the biological mechanism(s) for the less adaptive responses following the high rate strain protocol cannot be clearly explained by the present experimental design, we can argue that the reason may be related to the time of the applied mechanical loading. It is well accepted that cyclic strain affects the homeostasis of tendinous tissue (Kjaer, 2004; Wang and Thampatty, 2006; Wang et al., 2012). Initiated by the applied stress, the external strain of the tendon is transmitted through the extracellular matrix on the cytoskeleton of the tendon cells, which trigger cellular and molecular responses (e.g. synthesis of collagen and matrix proteins), affecting the mechanical and morphological tendon properties (Galloway et al., 2013; Heinemeier and Kjaer, 2011; Wang, 2006). The viscoelastic properties of the extracellular matrix (Wang, 2006) may influence the time-course of the external strain transmission to the tendon cells, indicating a time-dependent biological response. We suggest that the longer loading time during the reference protocol (3 s) may result in a more efficient transmission of the external tendon strain and, therefore, higher magnitude of strain on the tendon cells compared to the shorter loading times during the one-legged jumping (~0.26 s, high strain rate protocol). A possible greater transfer of the external tendon strain magnitude to the cellular level might be the reason for the superior adaptive responses of the tendon properties following the reference protocol. In agreement with the present findings, the results of our earlier studies (Arampatzis et al., 2010, 2007) showed a pronounced adaptation of the AT properties following an exercise intervention using a low strain frequency with longer strain duration per contraction compared to a high strain frequency with shorter strain duration per contraction (i.e., 0.17 Hz, 3 s loading/3 s relaxation vs. 0.5 Hz, 1 s loading/1 s relaxation). These findings indicate that increased hydrostatic pressure and fluid flow may not be as substantial for tendon adaptation compared to the duration of the repetitive tendon loading. To our knowledge the potential effect of strain rate, as an independent mechanical stimulus for tendon adaptation, has not been investigated so far on humans in vivo. In the present study one-legged jumps were used to increase the strain rate of the AT compared to a reference protocol of the same loading magnitude and volume. Studies investigating the effects of plyometric training on the AT properties found unchanged mechanical and/or morphological properties after an exercise intervention (Foure et al., 2012, 2011, 2010; Houghton et al., 2013; Kubo et al., 2007) or less adaptive responses compared to an isometric protocol featuring longer loading duration (Burgess et al., 2007). The above mentioned reports from the literature and the findings of the present study may indicate that a plyometric training using jumps does not provide an optimal mechanical stimulus for tendon adaptation compared to a training using longer durations of repetitive loading. However, the statistical tendency towards increased AT stiffness and average CSA as well as the effect size of 0.69 of the AT stiffness increase suggests that also a plyometric training may induce adaptive responses. These adaptations might became manifest, for instance, after a longer intervention duration as used in the present study (i.e., 14 weeks).

In the second intervention we found clear adaptations of the AT stiffness, which was a result of significant tendon hypertrophy and changes of the tendon material properties (i.e., increase in Young's modulus) in both protocols, demonstrating that the applied mechanical loading by means of the reference and the long strain duration protocol training effectively stimulated adaptive responses of the AT. Based on the viscoelastic properties of the extracellular matrix (Wang, 2006) and the time-dependent interaction between the extracellular matrix and the cytoskeleton, we proposed that longer strain duration (12 s vs. 3 s) may enhance the adaptation of mechanical and morphological tendon properties. However, the increase of AT stiffness and Young's modulus was more pronounced following the reference compared to the long strain duration protocol (AT stiffness: 54% vs. 25% and d = 1.51 vs. 0.68; Young's modulus: 45% vs. 18% and d = 1.31 vs. 0.57), indicating that the beneficial effect of the strain duration on tendon adaptation is limited and that repetitive loading in combination with an appropriate strain duration facilitate the adaptive response of tendons. It seems that the external tendon strain was effectively transmitted on the tendon cells during the 3 s loading, 3 s relaxation protocol (i.e., reference protocol) and that longer strain duration did not provide a superior adaptive effect. Therefore, we can argue that a certain duration of strain is a crucial component of an effective stimulus for tendon adaptation and under this premise can repetitive strain application provide advantageous adaptive responses compared to longer duration of static loading with fewer loading cycles. In accordance with this argumentation, Scott et al. (2011) reported a greater increase of tenocytes gene expression in vitro following cyclic (0.1 Hz) compared to static mechanical loading (5% strain) after 3 weeks in culture. This finding is in agreement with our in vivo experiments in which we applied a cyclic strain of 0.17 Hz (i.e., 3 s loading, 3 s relaxation) in the reference protocol that showed a greater adaptive response in comparison to long static loading. In a similar manner, a beneficial effect of repetitive loading compared to static was already reported regarding bone adaptation (Burr et al., 2002; Hert et al., 1971; Robling et al., 2001), giving further evidence for the superior effect of repetitive loading on the adaptive responses of biomaterials.

Beside a change in the Young's modulus (i.e., material properties) our results also revealed a significant increase in the CSA of the AT following the reference and long strain duration protocol training. Several studies in the past years reported increases in tendon cross-sectional area following long-term exercise-induced loading, indicating that hypertrophy is an important mechanism for tendon adaptation (Arampatzis et al., 2007; Couppe et al., 2008; Houghton et al., 2013; Kongsgaard et al., 2007; Magnusson and Kjaer, 2003; Rosager et al., 2002; Seynnes et al., 2009). Furthermore, the loading applied in the exercise protocols of the present study (i.e. 90% of maximal isometric force) was higher compared to other protocols (i.e. 70-80% of concentric one repetition maximum) reported in literature (Kongsgaard et al., 2007; Seynnes et al., 2009). With regard to the findings that tendon hypertrophy is dependent on the load magnitude during training (Arampatzis et al., 2007), we can argue that the stimulus applied in our experiments is effective in facilitating tendon hypertrophy. Furthermore, we can exclude fluid accumulation after the last training session being responsible for the higher tendon cross-sectional area found in the post measurements because a) the magnetic resonance images were recorded earliest 4 days after the final training bout, indicating an appropriate time for tissue recovery and b) the increase of tendon cross-sectional area was accompanied by an increase in Young's modulus, giving evidence for altered material properties (e.g. higher collagen content) independent of the cross-sectional area.

In all three protocols (i.e., reference, high strain rate and long strain duration), we aimed to induce a high strain magnitude of the AT by means of a target force level of 90% of the maximal voluntary isometric plantar flexion force, with regard to findings of our earlier experiments (Arampatzis et al., 2010, 2007). However, the individual strain magnitude was not controlled during the interventions and, thus, may not have been exactly the same for every participant. The data from our pre and post measurements show that the applied AT strain at 90% of the maximal voluntary isometric plantar flexion force was not significantly different between the experimental protocols ($p > 0.05$; intervention 1: reference 6.63 ± 1.24%, high strain rate 6.43 ± 1.18%; intervention 2: reference 6.49 ± 1.49%, long strain duration 6.94 ± 1.54% (mean strain of pre and post measurement ± SD)). Therefore, we suggest that individual deviations in strain magnitude did not affect our conclusions. Furthermore, the interventions were conducted using a homogeneous sample (i.e. healthy young adults) to avoid the influence of cofactors (e.g. age, gender). Thus, it still needs to be shown in how far our implications extend to other populations as for example female adults or elderly.

To investigate the effect of the four parameters of the mechanical stimulus (strain magnitude, strain frequency, strain rate and strain duration) on tendon adaptation by means of two parameter conditions (i.e. low and high) we conducted a total number of seven instead of 16 (4 parameters $\wedge$ 2 conditions) interventions. Because our first experiments (Arampatzis et al., 2010, 2007) showed that only the high strain magnitude protocols induced adaptive responses of the tendon, we decided to apply only the high strain magnitude condition in our present experimental design. Hence, the most effective training protocol we could identify in our first experiments (i.e. high strain magnitude and low frequency) was compared to a modulation of the strain rate and strain duration to prove a superimposed effect of these two parameters. By means of this systematic research approach we were able to reduce significantly the number of the necessary training protocols (from 16 to 7) without a decrease of the scientific quality.

The present results, in combination with our previous experiments (Arampatzis et al., 2010, 2007), demonstrate that a high strain magnitude beyond habitual loading must be applied to the tendon to induce adaptive responses of the mechanical, morphological and material tendon properties. A certain tendon strain duration (~3 s) seems to be necessary for an effective transmission of the external tendon strain on the cellular level and, therefore, plyometric exercises like jumping may not be an optimal training stimulus for tendon adaptation. Furthermore, the advantageous effect of longer tendon strain duration (i.e., >3 s) seems to be limited and repetitive application of strain provided a more effective stimulus for tendon adaptation in young healthy male adults.

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5.7 Conflict of interest

The authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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6. Main findings and conclusions

The present thesis aimed to provide enhanced knowledge about human tendon adaptation in vivo. Both experimental studies and the comprehensive literature analysis provided further evidence for the plasticity of tendons and deepened our understanding of the effect of the mechanical environment on the biology of the adaptive tendinous tissue. More precisely, the studies presented supported the importance of certain parameters of the mechanical strain in the context of controlled loading regimen as well as side-dependent daily loading patterns for tendon adaptation.

In summary, the present results showed that tendons respond to their mechanical environment, and adapt through alterations of their mechanical, material and morphological properties. Such adaptive responses were evidenced a) in the analysis of tendon properties of the non-dominant and dominant leg, showing significant differences as a potential result of a side-dependent loading pattern (first study), b) by the meta-analysis of recent intervention studies on tendon adaptation that revealed high intervention effect sizes on the tendon mechanical and material properties and a low effect size on the morphological properties (second study) and c) by the two exercise interventions that induced increases in tendon stiffness and Young's modulus as well as tendon hypertrophy (third study).

Compared to the morphological properties, the material properties (i.e. Young's modulus) of tendons seem to be more sensitive and instant in response to mechanical loading. The conducted meta-analysis of recent intervention studies showed that intervention-induced increases in tendon material properties were more pronounced compared to morphological changes, giving evidence for changes of material properties as a major and early adaptive mechanism of tendons. Furthermore, clear asymmetries of the Achilles tendon material but not of the cross-sectional area (i.e. morphological property) were found between the non-dominant and dominant leg in a normally active healthy population, most likely as a result of a higher sensitivity of the tendon material properties to the different daily loading pattern (i.e. foot preference) of both legs.

Further, the meta-analysis in particular but also the intervention study demonstrated clearly that the magnitude of the adaptive response strongly depends on the loading conditions and parameters of the applied mechanical strain. With regard to the effectiveness of certain loading parameters, both studies suggested a key role of loading magnitude for tendon adaptation. Furthermore, the controlled modulation of the rate and duration as additional main parameters of the mechanical strain by means of two separated exercise interventions (third study) indicated that a higher strain rate (~130 ms time to peak force) and longer strain duration (12 s) does not provide a superimposed effect for tendon adaptation compared to the reference protocol featuring a strain rate of approximately ~380 ms time to peak force, a strain duration of 3 s per contraction and repetitive applied loading. In combination with previous reported findings, it can, therefore, be concluded that a high strain magnitude beyond habitual loading must be applied to the tendon to induce adaptive responses of the mechanical, morphological and material tendon properties. Moreover, a minimum strain duration seems to be necessary to provide an effective stimulus, as shown by the results of the present studies as well as earlier controlled interventions. Thus, plyometric training like jumping with high strain rates but short loading times does not seem to be sufficient to induce significant adaptive responses of the Achilles tendon. The effect of longer duration of strain is, however, limited and repetitive strain application was a more effective stimulus for tendon adaptation.

The presented results provide valuable information on the crucial characteristics of an effective strain stimulus and general loading conditions to be considered for tendon adaptation with regard to the manipulation of tendon properties in the context of athletic performance and tendon injury prevention. From a methodological point of view, the results demonstrate that the effect of laterality of tendon properties should be taken into account in cross-sectional study designs as well as in clinical studies that use a comparison of both legs to detect pathological changes.

6.1 Practical implications

With regard to practical implications and recommendations, this thesis provides relevant information for the development of intervention strategies that aim to improve tendon properties as well as methodological considerations for study designs intending to assess tendon properties.

6.1.1 Training for the improvement of tendon properties

The results of the present intervention study in combination with the findings of previous studies show that a high strain magnitude repetitively applied over a certain duration per muscle contraction provided the most effective stimulus to improve the material and morphological tendon properties. Furthermore, a higher strain rate, or longer strain duration compared to the defined reference protocol, does not cause additional adaptations. Therefore, the following general training recommendations to induce adaptation of tendons can be formulated:

A training regimen for tendon adaptation needs to include the application of high muscle forces to provide an effective stimulus, because only high muscle forces induce the required tendon strain magnitudes.

The muscle contraction and, therefore, the associated tendon strain should be maintained for a certain duration (~3 s) to allow for an appropriate transmission of the external tendon strain on the cellular level.

Repetitive loading is more convenient than constant static loading to induce adaptive responses.

The application of high strain rates in combination with short loading durations like in plyometric training is less effective for tendon adaptation compared to isometric contractions featuring the mentioned characteristics.

Using dynamic exercises, the applied movement velocity should be low to allow the generation of high muscle forces (i.e. muscle force potential due to the force-velocity relationship). Eccentric muscle contractions feature a high muscle force potential and can be used to induce high strain magnitudes, but should be performed slowly and in a repetitive manner. Training using jumps to improve the tendon properties is less effective compared to repetitive loading with a certain duration. Furthermore, the meta-analysis on recent intervention studies indicated that a longer intervention duration of 14 weeks was beneficial compared to shorter ones, e.g. 8 weeks. Therefore, longer training regimen durations should be considered in the design of a specific tendon training.

6.1.2 Methodological considerations

The present thesis further provides recommendations for the measurement of tendon properties. The first study showed a clear asymmetry of the Achilles tendon properties between non-dominant and dominant leg in a normally active population. Therefore, a general transferability of the tendon properties of one leg to the contra-lateral one seems inappropriate. Consequently, to quantify the pathological or regenerative state of an affected leg in a clinical setting, it might be recommended to refer to the same side of matched control groups rather than the healthy contra-lateral leg because similar properties in a healthy state cannot be assumed per se. Furthermore, cross-sectional studies on tendon adaptation should consider potential differences of tendon properties between legs in their study design. Instead of measuring only one leg, it is to be recommended to either measure both legs or check for foot preference in advance to avoid possible effects of asymmetry on the sample comparison.

We reviewed and collated important aspects of an appropriate assessment of tendon mechanical, material and morphological properties as part of the included meta-analysis (second study; tab. 4.1). Experimental studies in the field of tendon research may take these methodological considerations into account.

6.2 Limitations

For the experimental studies included in the present thesis (first and third study), the mechanical (stiffness), material (Young's modulus) and morphological (cross-sectional area) properties of the Achilles tendon were examined combining dynamometry, magnetic resonance imaging and ultrasonography measurements. The following limitations regarding the measurement and calculation of the tendon properties can be stated.

First, the Achilles tendon stiffness and Young's modulus calculation based on the determination of the tendon force-elongation relationship during a maximum voluntary plantar flexion contraction. In the present study the tendon force was calculated from the ankle joint moment. Although the effects of inertia of the leg and dynamometer arm, axes misalignment, antagonistic muscle coactivation and passive forces of connective tissues have been considered in the tendon force calculation (see methods section of study one and three), additional synergistic muscles of the m. triceps surae contribute to the plantar flexion moment (e.g. m. tibialis posterior, m. flexor halucis longus, m. flexor digitorum longus, m. peroneus longus and brevis), affecting the calculation of the Achilles tendon force. To the best of my knowledge, no method exists to quantify the contribution of these synergists to the actual plantar flexion moment. However, these muscles feature relatively small cross-sectional areas and lever arms, indicating a minor contribution to the generated moment compared to the triceps surae muscles. Furthermore, this effect is consistent for all measurements and, thus, should not affect the conclusions of the studies.

To calculate the Achilles tendon lever arm, the tendon excursion method was used, i.e. ratio of the m. gastrocnemius medialis-Achilles tendon junction displacement to the corresponding angular excursion of the ankle joint. Although highly reliable (Fath et al., 2010), this method is based on the assumption that the tendon is rigid. Indeed, the tendon gets elongated when dorsal flexion increases (De Monte et al., 2006) and, therefore, the lever arm values may underestimate the true tendon lever arm values. However, the magnitude of tendon elongation due to the ankle angle change was shown to be very low in the range used for the lever arm calculation (i.e., 85-100 °, De Monte et al., 2006) and, therefore, the impact of non-rigidity should be negligible. Furthermore, the tendon lever arm values increase with higher muscle activation due to an alignment of the tendon (Maganaris et al., 1998, 2000). This change of the lever arm values was not directly measured in the present experiment but considered using suggested correction factors (Maganaris et al., 1998).

The Achilles tendon elongation during the maximum plantar flexion was measured by means of the displacement of the m. gastrocnemius medialis-Achilles tendon junction in the longitudinal axis, which was visualized using ultrasonography. This measurement included the elongation of the free Achilles tendon but also from the soleus aponeurosis. Since the aponeurosis may feature partly different elongation patterns than the free tendon, i.e. transverse strain (Azizi et al., 2009; Azizi and Roberts, 2009; Finni et al., 2003; Magnusson et al., 2003), this could have led to an underestimation of the actual tendon elongation. Further, for the calculation of the stress and, thus, the Young's modulus (i.e. material properties), the strain of the tendon-aponeurosis was related to the average cross-sectional area of the free Achilles tendon. The cross-sectional area of the soleus aponeurosis may differ from that of the free tendon, which could have affected the stress and Young's modulus calculations. However, these potential effects would be a systematic error that should not compromise the conclusions of the current studies.

6.3 New questions and future lines of research

The current thesis aimed to gain insight into the underlying mechanisms of tendon adaption. With regard to future studies, there are some open issues and proposed lines of research.

From a mechanobiological point of view, the findings of the present intervention studies in relation to earlier reports identified that a high strain magnitude, an appropriate strain duration (approximately 3 s) and repetitive loading are important mechanical characteristics of an effective stimulus to facilitate tendon adaptation. However, the underlying biological mechanisms that relates the cellular and molecular adaptive responses to strain duration, as well as to repetitive loading, remain unclear. It has been suggested that the transfer of the external tendon strain to the cells is time-dependent due to the viscous nature of the tendinous tissue and, thus, a certain time is necessary for an effective stimulus on the cells. In the present intervention study a strain duration of 3 s was efficient to induce significant tendon adaptive processes. Durations below (i.e. 0.26 s and 1 s (Arampatzis et al., 2010)) or beyond this interval (i.e. 12 s) did not induce comparable adaptations, indicating that the suggested duration is sufficient for the external strain transmission on the cellular level and that the effect of longer durations is limited. However, the argumentation of a time-dependent load transmission on the cell level remains speculative and needs to be supported by modelling studies, for example, and

in vitro evidence to understand how exactly strain duration acts on the different mechanotransduction pathways by which cells sense loading and affect the biological response.
Furthermore, to completely understand the effect of different strain durations for tendon adaptation further research is needed. In the current intervention the modulation of the strain duration was relatively high (3 s versus 12 s, factor 4) and durations in-between were not investigated, suggesting that durations longer than 3 s may also be effective. For example, in vitro experiments could modulate the strain duration more precisely using narrower intervals to assess the respective biological responses directly (e.g. collagen synthesis rate). In vivo studies of the effect of strain duration on acute and long-term tendon loading, using additional methodologies that assess cellular responses directly (e.g. microdialysis, tendon biopsies), may then be needed to prove if the findings of in vitro experiments are applicable to real life conditions.
Further, compared to the long strain duration protocol the load in the reference protocol was applied repetitively, i.e. one time 12 s and four times 3 s loading, 3 s relaxation, respectively. Since the adaptations were more pronounced following the repetitive loading, this difference in strain application (repetitive versus static) seems to influence the intervention-induced adaptive responses. The mechanobiological basis of repetitive versus static load application on tendon adaptive responses is yet unknown and needs further clarification. Under in vitro conditions controversial findings are reported, showing pronounced responses to either repetitive (Scott et al., 2011) or static (Feng et al., 2006) loading. Comparing static and repetitive loading intervention protocols, Kubo et al. (2009b) only reported increases in stiffness of the human patellar tendon following the static loading (Kubo et al., 2009b), indicating a dissimilar finding compared to the present results. However, the duration of the repetitive loading in this protocol was short (1 s), which may account for the lack of adaptation. Nevertheless, further research is necessary to clarify the effect and underlying mechanobiological mechanism of repetitive loading on tendon adaptation.
Regarding the effect of strain rate, the present intervention studies showed that a protocol with a higher strain rate - compared to the reference protocol using a lower one - did not induce significant adaptations. However, in the current experiments, the strain rate was increased by using one-legged jumps, which were associated with short strain durations (i.e. ground contact times of ~260 ms). As argued in the discussion section of study three, the short contact times may not have allowed for an appropriate transfer of the external strain stimulus on the cellular level. That means that the impact of short durations could have superimposed the potential effect of higher strain rates. Regarding the evidence of a facilitation of adaptive responses with higher strain rates (Lavagnino et al., 2008), future investigations are still needed to elucidate the separate effect of strain rate modulations.
Secondarily, although the present interventions promoted the identification of appropriate mechanical stimuli for tendon adaptation, the respective response may be affected by gender as

indicated by recent literature reports. For example, it has been shown that patellar tendon cross-sectional area was significantly increased in highly-trained male runners compared to matched controls in female counterparts (Magnusson et al., 2007), indicating that sex differences play a role in the adaptive responses of tendon morphological properties to habitual running-induced loading. Furthermore, biopsy studies suggested the presence of a sex influence in regard to the collagen synthesis rate following acute exercise. The results demonstrated that females respond less than males (Miller et al., 2007; Sullivan et al., 2009), most likely due to a depressing effect of circulating estrogen levels on the collagen synthesis (Magnusson et al., 2007). This means that the response to the mechanical stimuli on the cellular level may be sex-specific. As shown by the present meta-analysis, a direct comparison of tendon adaptive responses in vivo between male and female adults by means of controlled exercise interventions has not been conducted yet. Such information could deepen the understanding of sex-dependent tendon adaptive responses to mechanical loading and their potential influence on the higher tendon injury rates in women compared to men (Magnusson et al., 2007).

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I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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